The potential of zooprophylaxis and repellents against the insecticide resistant *Anopheles arabiensis* malaria vector in Ethiopia

Abebe Asale Gutto

Dissertation submitted in fulfillment of the requirements for the joint degree of Doctor of Philosophy (PhD) in Veterinary Sciences (Ghent University, Belgium) and Tropical and Infectious Diseases (Jimma University, Ethiopia)

2017

Promotor: Prof. Dr. Ir. Luc Duchateau
Department of Comparative Physiology and Biometrics
Faculty of Veterinary Medicine, University of Ghent
Salisburylaan 133, 9820 Merelbeke, Belgium

Promotor: Dr. Delenasaw Yewhalaw
Department of Medical Laboratory Sciences and Pathology
College of Health Sciences, Jimma University
Jimma, Ethiopia

To cite this thesis;

Abebe Asale (2017). The potential of zooprophylaxis and repellents against the insecticide resistant *Anopheles arabiensis* malaria vector in Ethiopia.

Dissertation submitted in fulfillment of the requirements for the joint degree Doctor of Philosophy (PhD) in Veterinary Sciences (Ghent University, Belgium) and Tropical and Infectious Diseases (Jimma University, Ethiopia)

Cover Design

The background picture of the cover page of the thesis depicts mosquito host preference (front cover) and the field experimental huts used (back cover)

Dedication

To my parents: Asale Gutto Gagga and Jatane Ennaro Boyyino

To my family: my wife Selamawit Wolde and my children Hermon Abebe and Basliel Abebe

Acknowledgments

First and foremost, I would like to thank my almighty God for keeping me safe and leading me all the way to the level of success where I am today. Second, I am so indebted to my promotors Prof. Dr. Ir. Luc Duchateau and Dr. Delenasaw Yewhalaw for their guidance, support, comment, encouragement and over all supervision of my PhD work which would have not been realized without their assistance.

I would like to thank Dr. Tibebu Habtewold (Imperial college of London, UK) for his critical comments especially sharing me his scientific expertise and technical advice in development of study design and refining the protocol. College of Natural Sciences, Tropical and Infectious Research center, Jimma University and Faculty of Veterinary medicine, Ghent University are acknowledged for allowing me to pursue my PhD training.

I would like to thank VLIR-IUC Program for funding my PhD project without which this study would have not been possible. I would like to thank VLIR-IUC Ghent program coordinating office as a hosting institute particularly Ms. Madina Abukeva and Ms. Mira Jashari, for their hospitality and support during my stay in Ghent. I would like to thank Mr. Andries Van den Broeck, Dr. Klaartje Goethals from the Biometry Department, Faculty of Veterinary Medicine, for their support and encouragement during my stays in Ghent University. At some point in my stay in Ghent I remember I was sick and everybody in the department was encouraging me. So thank you for your love and compassion that you have shown me. Particlarly Dries has stayed with me in the hospital almost half a day facilitating things which otherwise could have been very difficult to communicate in Dutch. I would like to thank also Ms. Aline Arens, my landlady who has shown incredible hospitality during my stay in Ghent. I would like to thank VLIR-IUC Jimma side also for the overall arrangement of my field work. They provided everything necessary for my field work including transportation, field laboratory equipments and chemicals, data collectors recruitment and payement arrangments. Particularly my sincere thank goes to Mr. Kasahun Eba, Mr. Boka Asefa, Mr. Esubalew Ayalew who have been coordinating all the necessary arrangements for my field work. Also my sincere appreciation to our field drivers and data clerks.

I would like to express my appreciation to all laboratory attendants and campus community of Sekoru Tropical and Infectious Diseases Research center for their help in providing lab space and necessary materials. I would like to thank the field entomological technicians (Mr. Mifta AbaGidi, Mr. Nasir Abarya, Mr. Abdo Jemal, Mr. Abdo AbaGidi, Mr. Workineh Jaleta and Mr. Abera Tesema (the late).

Finally, I would like to thank my wife Selamawit Wolde for all her encouragement and support without which I could not afford to accomplish alone. My children Hermon Abebe and Basliel

Abebe, I know I was too much occupied with my research work and could not give you enough time and love at the time when I am supposed to be with you. You are always my light and my hope that give all the encouragement to live and work. I love you so much.

Table of contents

List of acronyms

ACT	Artemisinin-Based Combination Therapy
DDT	Dichlorodiethyltrichloroethane
IRS	Indoor Residual Spray
LLINs	Long-Lasting Insecticidal Nets
ITN	Insecticide Treated Net
WHO	World Health Organization
CDC	Center for Disease Control and Prevention
GDP	Gross Domestic Product
RBM	Roll Back Malaria
FmoH	Federal Ministry of Health
ICIPE	International Center of Insect Physiology and Ecology
DEET	N, N-Di-ethyl-meta-toluamide
HLC	Human Landing Catch
HBI	Human Blood Index
BBI	Bovine Blood Index
CPT	Complete Protection Time
ANOVA	Analysis of Variance
RDT	Rapid Diagnostic Test

List of figures

List of tables

Chapter 1

General introduction

1.1 Malaria epidemiology

1.1.1 Global distribution and burden of malaria

After nearly a century since Laveran (Nye, 2002) described the plasmodium species and Ross (Rajakumar and Weisse, 1999) confirmed that female anopheline mosquitoes transmitted them, malaria remains a leading cause of morbidity and mortality worldwide. According to the World Malaria Report 2015, there were an estimated 214 million cases and 438,000 deaths of malaria in 2014, of which approximately 90% were in the African region (WHO, 2015). The global incidence of malaria (adjusted for population growth) was reduced by 60% between 2000 and 2015 and malaria mortality rates have decreased by 48% worldwide and by 54% in the African region (WHO, 2015).

The malaria parasites are one of the first pathogens to be studied in a public health context due to the high level of morbidity and mortality in humans (Rich and Xu, 2011). There are five prominent species of *Plasmodium* that cause disease in humans of which *Plasmodium falciparum* causes most mortality (Snounou et al., 1993). The different *Plasmodium* species are host specific though there have been periodic reports of simian malaria parasites being found in humans (Cox-Singh et al., 2008). *P. falciparum* and *P. vivax* are the most prevalent species worldwide. *P. falciparum* is generally confined to tropical and subtropical regions and is endemic in Africa, South and East Asia, South America, the Caribbean, and the Middle East, while *Plasmodium vivax* occurs in most of the temperate zones and also in large areas of the tropics, mostly in Asia and Latin America, and in some parts of Africa. The two other species *P. ovale* and *P. malariae* are less frequently encountered, and most commonly found in parts of Africa and Papua New Guinea. *Plasmodium knowlesi* is a parasite naturally occurring in several species of macaques in Southeast Asia but can be transferred to humans and cause disease (Galardo et al., 2009; Daily, 2006; Mendis et al., 2001; CDC, 2004).

Malaria is a disease of tropical and temperate countries between the latitudinal limits of 64° North and 32° South (Winstanley et al., 2004) with prevalence increasing towards the equator, and it is transmitted in areas where *Anopheles* mosquitoes can survive and multiply. Within these limits of latitude, there are large areas free of malaria making it essentially a focal disease, since the transmission of malaria depends greatly on the local environment and other conditions. The wide variation seen in the burden of malaria between different regions of the world is driven by several factors including the type and virulence of the prevalent parasite, the transmission capacity of the vector species and the susceptibility of the human population

(Pongsumpun and Tang, 2008). Tropical areas of the world have the most suitable combination of optimal rainfall, temperature and other factors allowing for breeding, feeding and survival of malaria vector mosquitoes. Thus, the *P. falciparum* parasite causing the most severe symptoms and the most efficient malaria vector mosquito *Anopheles gambiae* s.l. occur exclusively in tropical and subtropical parts of the world, especially in Africa (Pongsumpun and Tang, 2008; Adebote et al., 2008). Rainfall provides surface water in which female *Anopheles* can lay eggs. In arid areas where temperature is usually suitable, malaria transmission occurs only when rainfall provides temporary breeding habitats for vectors. These areas are often classified as "malarious near water" since transmission outside the rainy season typically occurs only along riverbeds, oases and other man-made surface water sites (Afrane et al., 2012; Cano et al., 2006).

Differences in the level of socio-economic development also contribute to regional and local variability in malaria burden. Determinants include poverty, quality of housing and access to health care, health education and existence of active malaria control programs. The poorest nations, where heavy malaria burden is found, generally have few resources for adequate control efforts. Therefore, malaria is endemic mostly in poor, tropical and subtropical areas of the world with children and pregnant women being at higher risk of malaria and more susceptible to severe disease (Hay et al., 2004; Costantini et al., 2009; Eve et al., 2005; Greenwood et al., 2005; Fils et al., 2010).

1.1.2 Malaria in Sub-Saharan Africa

According to WHO (2012) the vast majority of malaria deaths occurs in Africa, south of the Sahara, where malaria also presents a major obstacle to social and economic development. Malaria causes great economic loss in many African countries and is considered a major barrier to the socioeconomic development of the continent. Malaria has been estimated to cost Africa more than US$ 12 billion every year in lost gross domestic product (GDP), even though it could be controlled for a fraction of that sum (Hay et al., 2009; WHO, 2012).

Malaria kills an African child every 30 seconds and many children who survive an episode of severe malaria may suffer from learning impairments or brain damage. It is Africa's leading cause of mortality in children under five and constitutes 10% of the continent's overall disease burden. It accounts for 40% of public health expenditure, 30-50% of inpatient admissions, and up to 50% of outpatient visits in areas with high malaria transmission (Checchi et al., 2006; Barnes, 2009; WHO, 2010a; WHO, 2015).

Africa is the most affected due to a combination of factors including the presence of a very efficient mosquito vector (*An. gambiae*) and the predominant parasite species *P. falciparum*, which is the species that is most likely to cause severe malaria. Local weather conditions, which often allow transmission to occur year round, scarce resources and socio-economic instability, which have hindered efficient malaria control activities have also led to high malaria incidence. The malaria problem has aggravated due to the upcoming resistance of the malaria parasites against antimalarial drugs and the resistance of vectors against the most commonly used insecticides (Zhou et al., 2004; Erin et al., 2013; Plowe et al., 2007; Chrispinus et al., 2011).

Like other African countries, malaria is a major public health problem in Ethiopia with an average of 66 million or 68% of the total population being at risk and 26.4 million being at high risk with approximately 2.1 million cases recorded each year (FMoH, 2005; 2007; EPHI, 2011). The two main seasons for transmission of malaria in Ethiopia are September to December, the months that immediately follow the long rainy season and April to May, the months that follow the short rainy season that lasts from March to April (Ameneshewa, 1995; Baume et al., 2009; Alemu et al., 2011; Kenea, 2011). The two epidemiologically important malaria parasite species in the country are *P. falciparum* and *P. vivax* (O'Connor, 1967; Krasfur, 1977). The other two species, *P. malariae* and *P. ovale*, are also reported but less important epidemiologically (Gillies and De Meillon, 1968; Ribeiro et al., 1996; FMoH, 2004; Endeshaw et al., 2008; Tesfaye et al., 2011).

1.2 The malaria parasite and its vector

1.2.1 The biology and life cycle of the Plasmodium parasite

The malaria parasite has a complex life cycle involving both asexual and sexual stages with obligatory phases in both the human and the female *Anopheles* mosquito. In order to complete its life cycle, it has to infect the two hosts successively. The parasite gets its way into the human when infected mosquitoes inject it in the course of the blood meal. Once the parasite gets into the human bloodstream the sporozoites migrate to the liver cells, enter them and multiply asexually (schizogony). When the liver cell bursts, the schizonts are released into the bloodstream and invade the red blood cells (RBCs). The parasites grow inside the RBCs and eventually destroy them, which releases the daughter parasite merozoites that invade other RBCs. The blood stage parasites are those that cause the symptoms of malaria. It is during this stage that some of the merozoites develop into gametocytes that can be picked up by a female

Anopheles mosquito during a blood meal. Once the gametocytes get into the mosquito gut, they start another, different cycle of growth and multiplication in the mosquito (sporogonic cycle). The gametes develop into male and female sex cells and fuse to form zygotes in the insect's gut. The zygotes in turn become motile and elongated (ookinetes) and invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (Figure 1.1.) (Githinji et al., 2009; CDC, 2016).

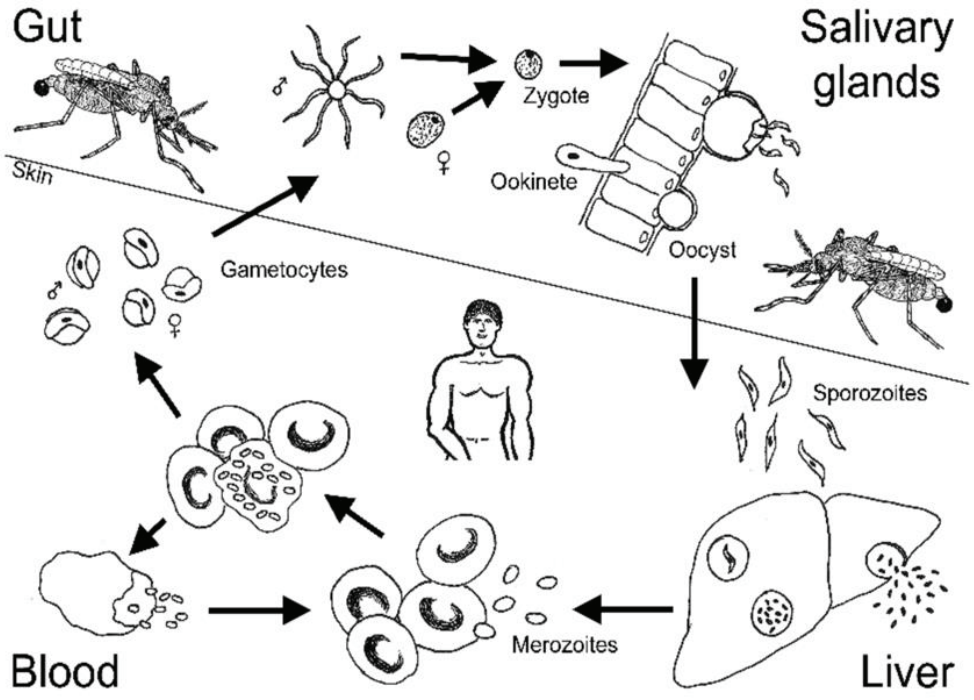

Figure 1.1 Life cycle of the *Plasmodium* parasite (Source: Githingji et al., 2009)

1.2.2 Biology and ecology of anopheline mosquitoes

The life cycle of *Anopheles* mosquitoes involves a complete metamorphosis and consists of four stages: the egg stage, the larval stage, the pupa and the adult stage (CDC, 2016). The lifespan of a female mosquito is approximately three to four weeks. Female *Anopheles* mosquitoes use sugar as energy source and also require a blood meal to complete the egg

development whereas the male *Anopheles* mosquito feeds exclusively on sugar from plants or other insects that feed on sugar from plants (CDC, 2016).

Adult females may lay 50 to 500 eggs per oviposition approximately two to four days after a blood meal. Anophelines, in contrast to other mosquito species, deposit single eggs onto the water surface, either by standing on the water surface or by hovering above it. The eggs contain lateral floats and are sensitive to desiccation. They hatch in two to three days (CDC, 2016; Manguin, 2008).

The larval stage consists of four instar phases. The larvae have no legs, a prominent head with mouth-parts and eyes, a broad thorax and a segmented abdomen. Anopheline larvae position themselves parallel to the water surface using specialized setae in order to breathe through spiracles located on the eighth abdominal segment. The larvae are browsers and collect food by feeding on algae, microorganisms and detritus in the water-air interface. Their habitat varies from unpolluted surface fresh water to ditches and the edges of small streams (Manguin, 2008; Becker et al., 2010).

The pupae are aquatic, comma-shaped and non-feeding. They float passively on the water surface while the process of metamorphosis takes place. The emergence of the adult takes approximately two days, depending on the temperature (Becker et al., 2010).

The duration from egg to adult varies according to the physico-chemical characteristics of the *Anopheles* breeding site (Rey, 2006). According to Oyewole et al. (2009), the pH, dissolved oxygen, temperature, ammonia, nitrate and phosphate concentrations all affect the larval development and survival as well as the rate of oviposition. The temperature has a large influence on the length of the gonotrophic cycle which shortens as the temperature increases, speeding up the larva-to-adult development, prolonging the larva and adult survival and increasing the biting rate (Oyewole et al., 2009; Afrane et al., 2005).

1.2.3 Malaria vectors and their global distribution

The human malaria parasite is transmitted by dipterans classified under the genus *Anopheles*. There are approximately 465 to 476 formally recognized species of *Anopheles* (Service, 2012; Sinka et al., 2012) out of which 70 are associated with the history of transmitting the human malaria parasite. Out of the 70 known vectors of the parasite, 41 are dominant vector species responsible for the majority of parasite transmission whereas the remaining 29 species have a minor role in the transmission (Hay et al., 2010; Sinka et al., 2012). There are several anopheline species that occur as a species complex, i.e., identical-looking species that can be

separated only by their chromosomal banding pattern by molecular methods (Service, 2012). A detailed knowledge of the global spatio-temporal distribution of the main *Anopheles* malaria vectors is a fundamental step in formulating regional and national vector control strategies.

The Neotropical zone is one of the regions where diverse *Anopheles* vector species are reported. Nine predominant and 10 secondary vector species distributed over 25 countries have been recorded from the region (Sinka et al., 2010). *An. albimanus, An. pseudopunctipennis, An. aquasalis, An. darlingi, An. marajoara, An. freeborni, An. Quadrimaculatus subgroup, An. Albitarsis complex and An. nuneztovari* are documented as predominant vector species whereas *An. cruzii, An. bellator*, *An. neivai, An. vestitipennis, An. neomaculipalpus, An. Nyssorhynchus braziliensis, An.* (*Nys*.) *triannulatus, An.* (*Nys*.) *strodei, An. Intermedius* and members of the *An.* (*Nys*.) *oswaldoi* complex are vector species documented with secondary role (Sinka et al., 2010; Service, 2012).

Most of the European countries were declared malaria free since the 1970s but the mosquitoes continue to exist in Europe, a phenomenon that is called anophelism without malaria (Fantini, 1994; Jetten and Takken, 1994).

On the other hand, the Middle East, particularly the Mediterranean region, continues to be suffering from malaria as the third largest burdened region following Africa and Asia (WHO, 2015). There are about 6 predominant vector species distributed over 49 different countries across Europe and the Middle East. These include *An. atroparvus*, *An. labranchiae*, *An. messeae*, *An. sacharovi*, *An. sergentii* and *An. superpictus* (Sinka et al., 2012). *An. messeae* remains the most dispersed vector species in terms of its geographic coverage across Europe and the Middle East extending from the United Kingdom in the west to Eastern Europe and into Asia. It is also the most northerly distributed vector of all species (Sinka et al., 2012).

The Indian subcontinent and the Asian Pacific are the second mostly affected regions following Africa with a 10% share of the global malaria burden (WHO, 2015). This region is also characterized by a high diversity of vector species and species complexes (Sinka et al., 2011). There are 19 dominant vector species including *An. minimus, An. punctulatus, An. sinensis, An. subpictus, An. sundaicus, An. barbirostris, An. culicifacies, An. dirus, An. farauti, An. fluviatilis, An. maculatus group, An. stephensi, An. aconitus, An. annularis, An. balabacensis, An. flavirostris, An. koliensis, An. lesteri* and *An. leucosphyrus* with the former 10 species belonging to a species complex with sibling species members that can be only identified via molecular techniques (Foley et al., 2007; Sinka et al., 2011).

In Africa, there are seven primary vector species (*An. gambiae, An. arabiensis, An. melas, An. merus, An. funestus, An. moucheti* and *An. nili*) recorded in 46 different countries (Sinka et al.,

2010). The species composition of the dominant vectors varies temporally and spatially in the continent (O'Connor, 1967). The former 4 in the aforementioned list previously belonged to the *Anopheles gambiae* complex but are now treated as separate species (Sinka et al., 2010). The *Anopheles gambiae* complex further includes four other less important member species, *An. bwambae*, *An. quadrianulatus* (formerly called *An. quadrianulatus* A), *An. amharicus* (formerly called *An. quadrianulatus* B) and *An. coluzzi* (formerly called *An. gambiae* M form) (Sinka et al., 2010; Coetzee *et al.,* 2013). It is confirmed that *An. funestus* and *An. nili* should be considered a species complex because of the presence of sibling member species within each group (Cohuet et al., 2003; Sinka et al., 2010).

In Ethiopia, *Anopheles arabiensis* is the principal vector of malaria covering all malarious areas of the country including north, south, east and west (Tulu, 1993). *An. pharoensis*, *An. funestus* and *An. nili* were documented as secondary vectors (Tulu, 1993; Taye et al., 2006; Massebo et al., 2015; Jaleta et al., 2016).

1.2.4 Malaria vector bionomics

1.2.4.1 Breeding site characteristics

Temperature, rainfall, relative humidity and altitude are the four major factors that affect the presence, abundance and seasonality of anopheline mosquitoes in a given area (Rogers et al., 2002; Hui et al., 2009). The quality of breeding sites and their distribution have a direct bearing on the mosquito population (Okara et al*.,* 2010). Physical factors (such as water temperature, light, water movement, turbidity, conductivity, vegetation, pH, soil type and salinity) and biotic interactions (such as predation and competition) are known to influence mosquito species assemblages. Mosquitoes often dominate in wetland ecosystems where suitable breeding sites are abundant and other physical factors are optimal for survival (Costantini et al., 2009; Chanda, 2010; Dery et al., 2010).

The degree of spatial heterogeneity and biotic interactions play an important role in determining how mosquito populations are structured (Shililu, 2001; Shililu et al., 2003). According to Galard et al. (2009) rainfall could be used to predict vector abundance if sufficient information on the biological and seasonal patterns of the vectors is available. The positive correlation between abundance and rainfall suggests that the presence of larval habitats influence adult abundance. On the other hand, a more recent study showed the presence of positive correlation between rainfall, abundance of the adult vector as well as malaria transmission (Oduola et al., 2012). Chanda (2011) also found that the dynamics and seasonal

abundance of malaria vectors is influenced by micro-ecology, rainfall and temperature patterns. These physical factors play a basic role in abundance and distribution of malaria vectors (Krasfur, 1977; Riberio et al., 1996; Kigadye et al., 2010; Afrane et al., 2012).

1.2.4.2 Feeding and resting behavior

Malaria vectors *An. gambiae, An. funestus* and *An. moucheti* are overwhelmingly known for their anthropophilic host preference behavior with occasional reports of zoophily, whereas *An. arabiensis*, *An. merus*, *An. melus* and *An. nili* are equally zoophilic and anthropophilic, readily feeding on both non-human vertebrate hosts and human hosts. The resting behavior of the mosquito is the behavior of the mosquito while digesting the blood meal by resting on walls or other resting places with poor lighting in the vicinity of the location where the blood meal was taken. With exception of *An. funestus*, which is reported as resting indoor (endophilic), all vector species are known to exhibit both endophilic and exophilic resting behavior (Sinka et al., 2010). *An. funestus*, *An. gambiae* and *An. moucheti* are mostly regarded as endophagic, i.e. feeding indoor, with respect to their feeding habit and are known to bite throughout the night. *An. arabiensis*, on the contrary, bites during the dusk and dawn period of the night and feeds both outdoor (exophagic) and indoor (endophagic).

1.2.4.3 Longevity and infectivity of Anophelines

The key parameters defining the vectorial capacity of mosquitoes are the longevity (Detinova, 1962) and the infectivity of vector mosquitoes (WHO, 2013). The former parameter refers to the life duration of the mosquito and the latter to the ability of a female vector mosquito to transmit malaria in its life span. Longevity and thus the survival rate can be estimated using the observed parity status of the female mosquitoes, i.e., the percentage parous females (Hugo et al., 2008; Detinova, 1962). A high proportion of nulliparous mosquitoes in a particular locality can be considered as an indicator of an emerging vector population.

Vector infectivity is obviously related to the incidence of malaria infection and the disease in human population (Beier et al., 1994). To transmit malaria, an individual *Anopheles* has to feed on humans at least twice, i.e., in the first episode it acquires an infection (infection of the vector by the parasite) and in the second episode it transmits the parasite (infection of human by the parasite). Obviously, vector longevity influences vector infectivity. A nulliparous mosquito cannot transmit malaria because it has not yet acquired the plasmodium parasite (Cook and Sinkins, 2010; Ghavami, 2005). On the other hand, a high proportion of parous mosquitoes

means that a large number of mosquitoes survives long enough for the *Plasmodium* parasite to complete the sporogonic cycle in the mosquito and make it infectious (Malainual et al., 1998).

1.3 Vector control

1.3.1 Long-lasting insecticidal nets and indoor residual spraying

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are pillars in malaria control strategies which target indoor feeding and resting vector species, aiming at either reducing the vector density or the infectivity rate of the vector (WHO, 2013). Indoor residual spray has been used since the 1950s (Biscoe et al., 2004) and LLIN was introduced in the 1990s (WHO, 1993; Jima et al., 2005) with the primary purpose of increasing community and personal protection. The proportion of the population sleeping under a LLIN has increased markedly in Sub-Saharan Africa, from less than 2% in 2000 to an estimated 46% in 2014 and 55% in 2015, with over half the population with access to an LLIN also protected by IRS in 2014 (WHO, 2015).

Because of the scaling up of both LLIN and IRS there has been a wide scale reduction of the malaria burden worldwide (WHO, 2015). According to a World Health Organization report, an estimated 663 million malaria cases were averted between 2001 and 2014 out of which 79% were due to vector control interventions LLINs and IRS (Bhatt et al., 2015; WHO, 2015). However, recent studies in the evolution of malaria control methods showed that the efficacy of both LLINs and IRS could be potentially compromised due to the presence of resistant vector populations (WHO, 2010b). On the other hand, given the fact that both LLINs and IRS are designed to provide protection against mainly indoor biting mosquitoes, residual transmission may sustain even with complete LLINs coverage due to the fact that it only targets indoor biting mosquitoes (Killeen, 2014). Thus, other complementary tools should be put in place in order to sustain the gains from LLINs/IRS and move towards the envisaged goal of malaria elimination (WHO, 2015).

In Ethiopia, studies around the Gilgel Gibe reservoir were initiated to investigate insecticide resistance and its underlying mechanisms. The *Anopheles* population around the Gilgel Gibe reservoir has developed resistance to DDT, deltamethrin and malathion, with the western kdr mutation as one of the underlying mechanisms (Yewhalaw et al., 2009; 2011). However, no field studies exist in Ethiopia to extrapolate the reported insecticide resistance to the effects it might cause on the efficacy of vector control methods LLINs and IRS. Therefore, we were

interested in our current PhD project to investigate whether the reported resistance has compromised the efficacy of LLINs and IRS.

1.3.2 Environmental management

Malaria vector control measures based on environmental management are non-toxic, cost-effective, and sustainable (Utzinger et al., 2001). According to the World Health Organization, environmental management for vector control includes the planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with man with a view of preventing or minimizing vector propagation and reducing the man-vector contact (WHO, 1982). Historically, environmental management for vector control has played a significant role in effectively reducing malaria in North Africa, America and Europe (Keiser et al., 2005). Yet, it almost disappeared following the invention of dichlorodiethyltrichloroethane (DDT), which was hailed as standardized single chemical intervention during the Global Malaria Eradication Campaign in the 1950s (Ault, 1994). It targets the immature stage of the vector before emergence, which is less prone to behavioral adaptation compared to the adult mosquito (Utzinger et al., 2001).

Environmental management strategies should include larval source management (habitat modification and source manipulation) (Imbahale et al., 2012), reduction of the human-vector contact through the strategic placement of settlements and better use of window screening (WHO, 2013). Habitat modification is the permanent alteration of the environment, i.e., the physical transformation of land, water or vegetation aimed at preventing, eliminating or reducing the larval breeding habitat with insignificant effect on the human environment (WHO, 1982; WHO, 2013). Larviciding is complementary to environmental management in which a suitable larvicide (synthetic or biological agent formulation) is applied to the breeding site using a correct dosage and appropriate formulation. The World Health Organization recommends 12 different insecticides including the biological agent formulation of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphericus* (Imbahale et al., 2012; WHO, 2013) to be used as larvicide in the control of mosquito larvae. The only drawback of larval source management is that it reduces malaria transmission in areas with well-defined breeding sites but less effectively in areas where breeding sites are uncertain and diffused (Fillinger et al., 2009).

On the other hand, as a component of environmental management, strategically placed animals can be used for diversion of blood-seeking mosquitoes away from humans (Mathys, 2010).

Due to the opportunistic feeding behavior of some mosquito species that feed readily on both human and on animals, the presence or introduction of animals into a community may affect the degree of human-vector contact, thereby potentially infectious bites to humans can be reduced. This strategy is called zooprophylaxis (WHO, 1982). Zooprophylaxis can be passive, active or insecticide augmented (Mathys, 2010). In passive zooprophylaxis cattle or other livestock possessed by the community could be associated with reduced risk of malaria whereas in active zooprophylaxis, livestock can be strategically placed in a deliberate attempt as a means of vector control (Bøgh et al., 2001; 2002). Insecticide zooprophylaxis involves the cattle sponging method by which insecticide is applied to domestic livestock topically using the animal dip method (Rowland et al., 2001; Mahande et al., 2007; Lyimo et al., 2012) or systemically by administering antihelminthics to the cattle (Fritz et al., 2009). Mosquitoes pick up a lethal dose when feeding on a treated animal.

Different studies were conducted in Ethiopia to determine the feeding behavior of vector mosquitoes through the assessment of the mosquito blood meal source (Habtewold et al., 2001; Animut et al., 2013; Massebo et al., 2015), and in the central and southern part of the country to determine the host preference of vector mosquitoes via human landing catch (Seyoum et al., 2002) and experimental traps (Habtewold et al., 2004; Tirados et al., 2006; 2011). However, the host preference assessment from the blood meal analysis could be biased because some hosts are more accessible than the other hosts. Moreover, the host preference varies from locality to locality. Thus, in this PhD project we investigate the host preference of *An. arabiensis*, the main malaria vector, in Southwestern Ethiopia using field and semi-field setups.

1.3.3 Repellents and other vector control methods

Personal protection remains one of the most effective strategies to minimize vector borne diseases (WHO, 2015). Long-lasting insecticidal nets and insecticide residual spraying are designed to tackle vector species that feed and rest indoor. Both methods do not protect against exophagic vectors, or those vectors that bite at times when people are not sleeping under their bed nets (Killeen et al., 2013). This may lead to a situation where mosquitoes which defy the existing control interventions may continue to sustain outdoor transmission. One good intervention for outdoor transmission could be the application of mosquito repellent. A mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages insects (and arthropods in general) from landing or probing on that surface (Patel et al., 2012). The usage of plant derived repellents has been practiced since ancient times (Peterson and

Coats, 2001) but the practice of using synthetic chemical repellents started at the end of world war-II when DEET was introduced in 1946 to be used in military personnel (Brown and Hebert, 1997).

Today there are many repellents both botanical and synthetic by origin. DEET has been considered the most broad-spectrum, efficacious insect repellent since the 1950s, serving as an effective repellent of mosquitoes and is currently available in concentrations ranging from 5% to 100%, although most products contain less than 40% (Katz et al., 2008). It is safe for use on cotton, wool, and nylon, although it has been found to damage spandex, rayon, acetate, pigmented leather and it may dissolve plastic (i.e., eyeglass frames) (Brown and Hebert, 1997). There are also plant derived essential oils such as Citronella oil (5%-15%) and Lemon eucalyptus oil (10%-30%) (Maia and Moore, 2011). The basic difference between synthetic repellents such as DEET and plant based essential oils is that synthetic repellents offer a longer time of protection (up to 8 hours) whereas plant based essential oils are relatively short-lived, need repeated application and offer protection of not more than an hour in most cases since they evaporate completely in a short time period (Patel et al., 2012).

In its renewed call for new tools and strategies to address residual transmission, the World Health Organization has recommended improving or developing novel vector interventions including repellents, house screening technologies, attractants to lure and trap/kill mosquitoes, topical or systemic insecticides applied on livestock aiming to kill mosquitoes that feed on the livestock (WHO, 2014). Despite their proven efficacy in personal protection, utilization of synthetic repellents such as DEET are less practiced in vulnerable communities in Africa due to lack of awareness (Govere et al., 2000; Mazigo et al., 2010), affordability (Sangoro et al., 2014) and health related risks (Katz et al., 2014).

Individual based studies with respect to the efficacy of repellents showed that topical repellents can protect from mosquito bites particularly in certain risk groups such as travelers, refugees and army personnel (Rowland et al., 2004, Kichen et al., 2009; Thrower and Goodyer, 2006; Lupi et al., 2013). However, the relevance of repellents as an intervention tool in community protection has been criticized following the large-scale community based cluster randomized trials. For instance, the combined treatment of 15% DEET and LLIN did not reduce the vector biting pressure as compared to LLIN only in Tanzania (Sangoro et al., 2014). Mass distribution of repellents (picaridin) in combination with LLINs did not have an effect on malaria incidence when compared with the control group in Cambodia, probably due to no adherence and inappropriate use of the repellents (Sluydts et al., 2016). A systematic review and meta-analysis of topical insect repellent efficacy against malaria endemic populations by Wilson et al. (2014)

did not show a significant reduction in *P. falciparum* and *P. vivax* malaria infection. Yet, to the best our knowledge, no study has been reported on the combined effect of repellent and zooprophylaxis. If combined, repellents and zooprophylaxis could offer better protection from infectious bites by diverting mosquitoes from human to a dead-end host/livestock.

In this PhD study we evaluated the efficacy of candidate repellents Mozigone developed by ICIPE, Kenya, Buzz off, a commercialized repellent from Ethiopia and DEET standard repellent, first using arm-in-cage laboratory experiments with further evaluation in a semi-field setup using experimental huts.

1.4 References

Adebote, A., Oniye J. and Muhammed, A. (2008). Studies on mosquitoes breeding in rock pools on in selbergs around Zaria, Northern Nigeria. *J. Vector Borne Dis.* 45, 21-28.

Afrane, Y.A., Lawson, B.W., Githeko, A.K. and Yan, G. (2005). Effects of microclimatic changes caused by land use and land cover on duration of gonotrophic cycles of *Anopheles gambiae* (*Diptera: Culicidae*) in western Kenya highlands. *J. Med. Entomol.* 42, 974-980.

Afrane, Y., Githeko, A. and Yan, G. (2012). The ecology of Anopheles mosquitoes under climate change: case studies from the effects of deforestation in East African highlands. *Ann. N.Y. Acad. Sci.* 1249, 204-210.

Alemu, A., Tsegaye, W., Golassa, L. and Abebe, G. (2011). Urban malaria and associated risk factors in Jimma town, south-west Ethiopia. *Malar. J.* 10, 173.

Ameneshewa, B. (1995). The behavior and biology of *Anopheles arabiensis* in relation to epidemiology and control of malaria in Ethiopia [PhD. Thesis]. University of Liverpool, U. K., pp 288.

Ault, S.K. (1994). Environmental management: A re-emerging vector control strategy. *Am. J. Trop. Med. Hyg.* 50, 35-49.

Ayala, D., Costantini C., Ose K., Kamdem, G.C., Antonio-Nkondjio, C., Agbor J., Awono-Ambene P., Fontenille D. and Simard F. (2009). Habitat suitability and ecological niche profile of major malaria vectors in Cameroon. *Malar. J.* 8, 307.

Barnes, K.I., Chanda, P. and Barnabas, G. (2009). Impact of the large-scale deployment of artemether/lumefantrine on the malaria disease burden in Africa: case studies of South Africa, Zambia and Ethiopia. *Malar. J.* 8, 145-158.

Baume, C.A., Reithinger, R. and Woldehanna, S. (2009). Factors associated with use and non-use of mosquito nets owned in Oromia and Amhara Regional states, Ethiopia. *Malar. J.* 8, 258-264.

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C. and Kaiser, A. (2010). *Mosquitoes and their control.* 2nd ed. Springer-Verlag Berlin Heidelberg, 9-22.

Beier, C., Oster, N., Onyango, K., Bales, D., Sharwood, A., Perkins, V., Koech, D., Whitmire, E., Diggs, L. and Hoffman, L. (1994). *Plasmodium falciparum* incidences relative to entomological inoculation rates at a site proposed for testing malaria vaccines in Western Kenya. *Am. J. Trop. Med. Hyg.* 50, 529-36.

Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Briët, O., Penny, M.A., Smith, T.A.,

Bennett, A., Yukich, J., Eisele, T.P., Griffin, J.T., Fergus, C.A., Lynch, M., Lindgren, F., Cohen, J.M., Murray, C.L.J., Smith, D.L., Hay, S.I., Cibulskis, R.E. and Gething, P.W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207.

Biscoe, M.L., Mutero, C.M. and Kramer, R.A. (2004). Current policy and status of DDT use for malaria control in Ethiopia, Uganda, Kenya and South Africa. Sri Lanka: international water management institute.

Bogh, C., Clarke, S.E., Pinder, M., Sanyang, F. and Lindsay, S.W. (2001). Effect of passive zooprophylaxis on malaria transmission in the Gambia. *J. Med. Entomol.* 38, 822-828.

Bogh, C., Clarke, S.E., Walraven, G.E. and Lindsay, S.W. (2002). Zooprophylaxis, artefact or reality? A paired-cohort study of the effect of passive zooprophylaxis on malaria in the Gambia. *Trans. Roy. Soc. Trop. Med. Hyg.* 96, 593-596.

Brown, M. and Hebert, A.A. (1997). Insect repellents: an overview. *J. Am. Acad. Dermatol.* 36, 243-249.

Cano, J., Descalzo, M., Moreno, M., Chen, Z., Nzambo, S., Bobuakasi, L., Buatiche, J., Ondo, M., Micha, F and Benito, A. (2006). Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). *Malar. J.* 5, 21.

CDC (2004). *Malaria: geographic distribution and epidemiology*. Centers for disease control and prevention, Atlanta, U.S.A, 56pp.

CDC (2016). Centre for Disease Control and Prevention. About malaria Biology: Mosquitoes http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html (accessed at 04/28/2016)

Chanda, E. (2011). Optimizing impact assessment of entomological intervention for malaria control in an operational setting in Zambia. Ph.D. dissertation, Liverpool University, England, pp 1-175.

Checchi, F., Cox, J., Balkan, S., Tamrat, A., Priotto, G., Alberti, K.P., Zurovac, D and Guthmann, J.P. (2006). Malaria epidemics and interventions, Kenya, Burundi, Southern Sudan, and Ethiopia. *Emerg. Infect. Dis.* 12, 1477-1485.

Chrispinus, M., Donald, S., Moses, N. and John, V. (2011). Targeted indoor insecticide and malaria control in the western highlands of Kenya. *J. Infect. Dis. Immun.* 3, 50-58.

Coetzee, M., Hunt, R., Wilkerson, R., Torre, A., Coulibaly, M. and Besansky N. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* 3619, 246-274.

Cohuet, A., Simard, F., Toto, J., Kengne, P., Coetzee, M. and Fontenille, D. (2003). Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species. *Am. J. Trop. Med. Hyg.* 69, 200-205.

Cook, P. and Sinkins, S. (2010). Transcriptional profiling of *Anopheles gambiae* mosquitoes for adult age estimation. *Insect Mol. Biol.* 19, 745-751.

Costantini, A., Ose, K., Kamdem, G., Antonio-Nkondjio, C., Agbor, J., Awono-Ambene, P., Fontenille, D. and Simard, F. (2009). Habitat suitability and ecological niche profile of major malaria vectors in Cameroon. *Malar. J.* 8, 307-322.

Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J. and Singh B. (2008). *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin. Infect. Dis.* 46, 165-171.

Daily, J. P. (2006). Antimalarial drug therapy: the role of parasite biology and drug resistance. *J. Clin. Pharmacol.* 52, 1487-1497.

Dery, D., Brown, C., Asante, K., Adams, M., Dosoo, D., Amenga-Etego, S., Wilson, M., Chandramohan, D., Greenwood, B. and Owusu-Agyei, S. (2010). Patterns and seasonality of malaria transmission in the forest-savannah transitional zones of Ghana. *Malar. J.* 9, 314.

Detinova, S. (1962). Age grading methods in *Diptera* of medical importance with special reference to some vectors of malaria. Monograph Series, *World Health Org* 47:1-216

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P.M., Shargie, E.B., Ejigsemahu, Y., Ayele, B., Yohannes, G., Teferi, T., Messele, A., Zerihun, M., Genet, A., Mosher, A.W, Emerson, P.M. and Richards, F.O. (2008). Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malar. J.* 7, 115-118.

EPHI. (2012). Ethiopian public health institute. Ethiopia national malaria indicator survey 2011. Available from: www.unicef.org/ethiopia/ET_MIS_2011_Report.pdf [accessed: 11/1/2016]

Erin, A., Mordecai, E.A., Paaijmans, K.P., Johnson, L.R., Balzer, C., Tal, B., Moor, E., McNally, A., Pawar, S., Ryan, S.J., Smith, T.C. and Lafferty K.D. (2013). Optimal temperature for malaria transmission is dramatically lower than previously predicted**.** *Ecol. Lett.* 16, 22-30.

Eve, W., Suprotik. B., and Kara, H. (2005)**.** Is malaria a disease of poverty? *Trop. Med. Int. Health* 10, 1047-1059.

Fantini, B. (1994). Anophelism without malaria: an ecological and epidemiological puzzle. *Parasitologia* 36, 83-106.

Fillinger, U., Sombroek, H., Majambere, S., van Loon, E., Takken, W. and Lindsay S.W. (2009). Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. *Malar. J.* 8, 62.

Fils, E., Ntonga, P., Belong, P. and Messi, J. (2010). Contribution of mosquito vectors in malaria transmission in an urban district of Southern Cameroon. *J. Entomol. Nematol.* 2, 13-17.

FMoH (2004). Malaria diagnosis and treatment guidelines for health workers in Ethiopia. 2nd edi. Addis Ababa, Ethiopia.

FMoH (2005). Malaria control in Ethiopia. Malaria and other Vector borne Diseases Prevention and Control Team. Africa Malaria Day; 58pp.

FMoH (2007). Africa malaria day 2007 celebrations in Ethiopia, joint press release for the commemoration of the Africa malaria day; 1-98pp.

Foley, D.H., Rueda, L.M. and Wilkerson, R.C. (2007). Insight into global mosquito biogeography from country species records. *J. Med. Entomol.* 44, 554--567.

Fritz, M.L., Siegert, P.Y., Walker, E.D., Bayoh, M.N., Vulule, J.R. and Miller, J.R. (2009). Toxicity of blood meals from ivermectin-treated cattle to *Anopheles gambiae s.l.* *Anna Trop Med Parasitol* 103, 539-547.

Galardo, A., Zimmerman, O., Lounibos, L., Young, L., Gardo, C., Arruda, M. and Couto, A. (2009). Seasonal abundance of anopheline mosquitoes and their association with rainfall and malaria along the Matap River, Amap, Brazil. *Med. Vet. Entomol.*23, 335-349.

Ghavami, M. (2005). Estimation and Comparison of *Anopheles maculipennis s.l.* (Diptera: Culicidae) Survival Rates with Light-trap and Indoor Resting Data. *Iran J. Publ. Health* 34, 48-57.

Gillies, T. and De Meillon, B. (1968). The anopheline of Africa south of the Sahara (Ethiopian zoogeographical region). Johannesburg: The South African institute for medical research

Govere, J.M., Durrheim, D.N., La Grange, J.J.P., Mabuza, A. and Booman, M. (2000). Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga province South Africa. *South Afri. Med. J.* 90, 611-616.

Greenwood, B.M., Bojang, K., Whitty, C.J. and Targett, G.A. (2005). Malaria. *Lancet Infect. Dis.* 365, 1487-1498.

Hay, S.I., Guerra, C.A., Gething, P.W., Patil, A.P., Tatem, A.J., Noor, A.M., Kabaria, C.W., Manh, B.H., Elyazar, I.R., Brooker, S., Smith, D.L., Moyeed, R.A. and Snow, R.W. (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLos Med.* 6, e1000048.

Hay, S.I., Sinka, M.E., Okara, R.M., Kabaria, C.W., Mbithi, P.M., Tago, C.C., Benz, D., Gething, P.W., Howes, R.E., Patil, A.P., Temperley, W.H., Bangs, M.J., Chareonviriyaphap, T., Elyazar, I.R., Harbach, R.E., Hemingway, J., Manguin, S., Mbogo, C.M., Rubio-Palis, Y. and Godfray H.C. (2010). Developing global maps of the dominant Anopheles vectors of human malaria. *PLos Med.* 7, e1000209.

Hay, S., Guerra C., Tatem A., Noor A. and Snow R. (2004). The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect. Dis.* 4, 327-336.

Hui F., Chen Z., Cheng X , Liang L., Huang H., Fang L., Yang H., Zhou H., Yang H., Zhou X., Cao, W. and Gong P. (2009). Spatio-temporal distribution of malaria in Yunnan Province, China. *Am. J. Trop. Med. Hyg.* 81, 503-509.

Hugo, E., Quick-Miles, A., Kay, H. and Ryan, A. (2008). Evaluations of mosquito age grading techniques based on morphological changes. *J. Med. Entomol.* 45, 353-369.

Imbahale, S.S., Githeko, A., Mukabana, W.R. and Takken, W. (2012). Integrated mosquito larval source management reduces larval numbers in two highland villages in western Kenya. *BMC Public Health* 12, 362.

Jaleta, T.K., Hill, R.S., Birgersson, G., Tekie, H. and Ignell, R. (2016). Chicken volatiles repel host-seeking malaria mosquitoes. *Malar. J.* 15, 354.

Jima, D., Tesfaye, G., Deressa, W., Woyessa, A., Kebede, D. and Alamirew, D. (2005). Baseline survey for the implementation of insecticide treated mosquito nets in malaria control in Ethiopia. *Eth. J. Health Dev.* 19, 16-23.

Kashiwada, M. and Ohta, S. (2010). Modeling the spatio-temporal distribution of the anopheles mosquito based on life history and surface water conditions. *Open Ecol. J.* 3, 29-40.

Katz, T.M., Miller, J.H. and Hebert, A.A. (2008). Insect repellents: historical perspectives and new developments. *J. Am. Acad. Dermatol.* 58, 865-71.

Keiser, J., Singer, B.H. and Utzinger, J. (2005). Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect. Dis.* 5, 695-708.

Kenea, O., Balkew, M. and Gebre-Michael T. (2011). Environmental factors associated with larval habitats of anophelines mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the rift valley, central Ethiopia. *J. Vector Borne Dis.* 48, 85-92.

Kigadye, E., Nkwengulila, G., Magesa, S. and Abdulla, S. (2010). Diversity, spatial and temporal abundance of *Anopheles gambiae* complex in the Rufiji River basin, south-eastern Tanzania. *Tanzania J. Health Res.* 12, 1-6.

Killeen, F.G. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar. J.* 13, 330.

Killeen G.F., Seyoum A., Sikaala C., Zomboko A.S., Gimnig J.E., Govella N.J. and White M.T. (2013). Eliminating malaria vectors. *Parasites Vectors* 6, 172.

Kitchen, L. W., Lawrence, K. L. and Coleman, R. E. (2009), The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. *J. Vector Ecol.* 34, 50–61. doi:10.1111/j.1948-7134.2009.00007.x

Korgaonkar, N., Kumar, A., Yadav, R., Kabadi, D. and Dash, A. (2012). Mosquito biting activity on humans & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. *Indian J. Med. Res.* 135, 120-126.

Krasfur, E. (1977). The bionomics and relative prevalence of anopheles species with respect to the tramsmission of plasmodium to man in western Ethiopia. *J. Med. Entomol.* 14, 180-194.

Lupi, E., Hatz, C. and Schilgenhauf, P. (2013). The efficacy of repellents against *Aedes, Anopheles, Culex* and *Ixodes* spp. *Travel Med. Infect. Dis.*11, 374-411. http://dx.doi.org/10.1016/j.tmaid.2013.10.005

Lyimo, I.N., Ng'habi, K.R., Mpingwa, M.W., Daraja, A.A., Mwasheshe, D.D., Nchimbi, N.S., Lwetoijera, D.W. and Mnyone, L.L. (2012). Does cattle milieu provide a potential point to target wild exophilic *Anopheles arabiensis* (Diptera: Culicidae) with entomopathogenic fungus? A bio insecticide zooprophylaxis strategy for vector control. *J. Parasitol. Res.* doi:10.1155/2012/280583.

Mahande, A.M., Mosha, F.W., Mahande, J.M. and Kweka, E.J. (2007). Role of cattle treated with deltamethrine in areas with a high population of *Anopheles arabiensis* in Moshi, northern Tanzania. *Malar. J.* 6, 109.

Maia, M.F. and Moore, S.J. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. *Malar. J.* 10, S11.

Malainual, N., Thavara, U., Chansang, C. and Mogi, M. (1998). Estimation of gonotrophic cycle lengths and survival rates for vector mosquitoes of Japanese encephalitis in the suburbs of Bangkok, Thailand, *J. Med. Entomol. Zool.* 49, 105 -112.

Manguin, S. (2008). Biodiversity of malaria in the world. John Libbey Eurotext Paris, p.70.

Massebo, F., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2015). Zoophagic behavior of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasites Vectors* 8, 645.

Mathys, T. (2010). Effectiveness of zooprophylaxis for malaria prevention and control in settings of complex and protracted emergency. *Resilience: Interdiscipl. Perspect. Sci. Humanitarianism* 1, 1-26.

Mazigo, H.D., Obasy, E., Mauka, W., Manyiri, P., Zinga, M., Kweka, E.J., Mnyone, L.L. and Heukelbach, J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. *Malar. Res. Treat. 2010*, 794261

Mendis, K., Sina, B.J., Marchesini, P. and Carter, R. (2001). The neglected burden of *P. vivax* malaria. *Am. J. Trop. Med. Hyg.* 64, 97-106.

Mereta, T.S., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, O.S., Legesse, W. and Goethals, L.M.P. (2013). Physico-chemical and biological characterization of Anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites Vectors* 6, 320.

Muturi, E.J., Mwangangi, J., Shililu, J., Jacob, B.G., Mbogo, C., Githure, J. and Novak, R.J. (2008). Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. *J. Vector Ecol.* 33, 56-63.

O'Connor, C. (1967). The distribution of anopheline mosquitoes in Ethiopia. *Mosq News.* 27, 42-54.

Nye, E.R. (2002). Alphonse Laveran (1845-1922): discoverer of the malarial parasite and Nobel laureate, 1907. *J. Med. Biogr.* 10, 81-87.

Oduola, A., Otubango, O., Olojele, J., Oyewole, I. and Awolola, T. (2012). Transmission risk indices of three Anopheles species in selected rural communities of Oyo southwestern Nigeria. *Malar. J.* 7, 142-48.

Okara, R., Sinka, M., Minakawa, N., Mbogo, C., Hay, S. and Snow, R. (2010). Distribution of the main malaria vectors in Kenya. *Malar. J.* 9, 69.

Oyewole, I.O., Momoh, O.O., Anyasor, G.N., Ogunnowo, A.A., Ibidapo, C.A., Oduola, O.A., Obansa, J.B. and Awolola, T.S. (2009). Physico-chemical characteristics of Anopheles breeding sites: Impact on fecundity and progeny development. *Afr. J. Env. Sci. Technol.* 3, 447-452.

Patel, E.K., Gupta, A. and Oswal, R.J. (2012). A Review on: mosquito repellent methods. *Int. J. Pharm. Chem. Boil. Sci.* 2, 310-317.

Peterson, C. and Coats, J. (2001). Insect repellents past, present and future. *Pestic Outlook* 12,154-8.

Plowe, C.V., Roper, C., Barnwell, J.W., Happi, C.T., Joshi, H.H., Mbacham, W., Meshnick, S.R., Mugittu, K., Naidoo, I., Price, R.N., Shafer, R.W., Sibley, C.H., Sitherland, C.J.,

Zimmerman, P.A. and Rosenthal, P.J. (2007). World antimalarial resistance network (WARN) III: molecular markers for drug resistant malaria. *Malar. J.* 6, 112-121.

Pongsumpun P. and Tang I.M. (2008). The transmission dynamics of *Plasmodium vivax* malaria at the local level. Ladkrabang, Thailand: 406-410pp.

Rajakumar, K. and Weisse, M. (1999). Centennial year of Ronald Ross' epic discovery of malaria transmission: an essay and tribute. *South Afr. Med. J.* 92, 567-571.

Rey, J.R. (2006). *The mosquito.* Entomology and nematology department, Florida cooperative extension service, institute of food and agricultural sciences, University of Florida. 727.

Ribeiro, J., Seulu, F. Abose, T., Kidane, G. and Teklehaimanot, A. (1996). Temporal and spatial distribution of anopheline mosquitos in an Ethiopian village: implications for malaria control strategies. *Bull. World Health Organ.* 74, 299-305.

Rich, S.M. and Xu, G. (2011). Resolving the phylogeny of malaria parasites. *Proc. Natl. Acad. Sci. U.S.A.* 108, 12973-12974.

Rogers, J., Randolph, S., Snow, R. and Hay, S. (2002). Satellite imagery in the study and forecast of malaria. *Nature* 415, 710-715.

Rowland, M., Durrani, N., Kenward, M., Mohammed, N., Urahman, H. and Hewitt, S. (2001). Control of malaria in Pakistan by applying deltamethrin insecticide to cattle: a community-randomized trial. *Lancet* 357, 1837-1841.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J. and Fayaz, M. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop. Med. Int. Health*, 9, 335–342. doi:10.1111/j.1365-3156.2004.01198.x

Sangoro, O., Lweitojera, D., Simfukwe, E., Ngonyani, H., Mbeyela, E., Lugiko, D., Kihonda, J., Maia, M. and Moore, S. (2014). Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data. *Malar. J.* 13, 159.

Schellenberg, D., Menendez, C., Aponte, J.J., Kahigwa, E., Tanner, M. and Mshinda, H. (2005). Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomized, placebo-controlled trial. *Lancet Infect. Dis.* 365, 1481-1483.

Service, M. (2012). *Medical entomology for students*. 5th ed. New York: Cambridge University press.

Shililu, J. (2001). Malaria vector studies in Eritrea. EHP (environmental health project) USAID Washington, USA, pp 1-44.

Shililu, J., Ghebremeskel, T., Mengistu, S., Fekadu, H., Zerom, M., Mbogo, C., Githure, J., Gu W., Novak, R. and Beier, J. (2003). Distribution of anopheline mosquitoes in Eritrea. *Am. J. Trop. Med. Hyg.* 69, 295-302.

Sinka, M.E., Rubio-Palis, Y., Manguin, S., Patil, A.P., Temperley, W.H., Gething, P.W., Van Boeckel, T., Kabaria, C.W., Harbach, R.E. and Hay, S.I. (2010). The dominant Anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. *Parasites Vectors* 3, 72-10.

Sinka, M.E., Bangs, M.J., Manguin, S., Chareonviriyaphap, T., Patil, A.P., Temperley, W.H., Gething, P.W., Elyazar, I.R., Kabaria, C.W., Harbach, R.E. and Hay, S.I. (2011). The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. *Parasites Vectors* 4, 89.

Sinka, M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Burkot, T.R., Harbach, R.E. and Hay S.I. (2012). A global map of dominant malaria vectors. *Parasites Vectors* 5, 69.

Sluydts, V., Durnez, L., Heng, S., Gryseels, C., Canier, L., Kim, S., Van Roey, K., Kerkhof, K., Khim, N., Mao, S., Uk, S., Sovannaroth, S., Grietens, K.P., Sochantha, T., Menard, D. and Coosemans M. (2016). Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomized controlled trial. *Lancet Infect. Dis.* 16, 1169-1177.

Snounou, G., Viriyakosol, S., Jarra, W., Thaithong, S. and Brown, K.N. (1993). Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol. Biochem. Parasitol.* 58*,* 283-292.

Taye, A., Hadisa, M., Adugnaa, N., Tilahuna, D. and Wirtzb, R.A. (2006). Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis* from Sille, Ethiopia. *Acta Trop.* 97, 50-54.

Tesfaye, S., Belyhun, Y., Teklu, T., Mengesha, T. and Petros, B. (2011). Malaria prevalence pattern observed in the highland fringe of Butajira, Southern Ethiopia: a longitudinal study from parasitological and entomological survey. *Malar. J.* 10,153.

Thrower, Y. and Goodyer, L. I. (2006). Application of insect repellents by travelers to malaria endemic areas. *J. Travel Med.* 13, 198–202. doi:10.1111/j.1708-8305.2006.00051.x

Trape, J., Pison, G., Spiegel, A., Enel, C. and Rogier, C. (2002). Combating malaria in Africa. *Trends Parasitol.* 18, 224-230.

Tulu, A.N. (1993). *Malaria.* In: Kloos H. and Zein A.Z. (Eds.), The ecology of health and disease in Ethiopia, Westerview Press, Boulder pp. 341-352.

Utzinger, J., Tozan, Y. and Singer, B.H. (2001). Efficacy and cost-effectiveness of environmental management for malaria control. *Trop. Med. Int. Health* 6, 677-687.

Van Lieshouta, M., Kovatsb, R.S., Livermorec, M.T.J. and Martensa, P. (2004). Climate change and malaria: analysis of the SRES climate and socio-economic scenarios. *Glob. Environ. Change* 14, 87-99.

WHO (1982). *Manual on environmental management for mosquito control with special emphasis on malaria vectors.* WHO Offset Publication 1982; 66. Available from: http://whqlibdoc.who.int/ publications/1982/9241700661_eng.pdf [accessed on: 09/12/2016]

WHO (1993). *WHO study group on the implementation of the global Plan of action for malaria control.* [meeting held in Geneva from 8 to 12 February 1993]. WHO Technical Report Series 1993;839.

WHO (2010a). What is malaria? United nations decade to roll back malaria. World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland 2010; p. 2-4

WHO (2010b). World health organization. *Coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control.* WHO Press, World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland 2010; p. 1-46.

WHO (2012). World health organization malaria report 2012. World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland 2012; p. 1-276.

WHO (2013). World malaria report. World health organization. Geneva. [Online] Available from http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/ [accessed on: 04/04/2016]

WHO (2013). Malaria entomology and vector control guide for participants. World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland 2012; p. 1-190.

WHO (2013). *Larval Source management: a supplementary measure for malaria vector control: An Operational Manual.* [Online] Available from http://apps.who.int/iris/bitstream/10665/85379/1/9789241505604_eng.pdf [accessed on: 09/12/2016]

WHO (2014). Control of residual malaria parasite transmission: guidance note. [Online] Available from WHO/HTM/GMP/MPAC/2014.5 [accessed on: 07/25/2016]

WHO (2015). World malaria report. World health organization. Geneva. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed on: 09/09/2016]

Wilson, A.L., Chen-Hussey, V., Logan, J.G. and Lindsay S.W. (2014). Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. *Malar J.* 13, 446. DOI: 10.1186/1475-2875-13-446

Winstanley, P., Ward S., Snow R. and Breckenridge A. (2004). Therapy of *Falciparum* malaria in Sub-Saharan Africa: from molecule to policy. *Clin Microbiol Rev.* 17, 612-637.

Yewhalaw, D, Wassie, F, Steurbaut, W, Spanoghe, P, Van Bortel, W, Denis, L., Tessema, D.A., Getachew, Y., Coosemans, M., Duchateau, L. and Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PLoS One* 6, 1 e16066.

Zhou, G., Minakawa, N., Githeko, A.K. and Yan G. (2004). Association between climate variability and malaria epidemics in the east African highlands**.** *Proc. Natl. Acad. Sci. U.S.A.* 101, 2375-2380.

Chapter 2

Objectives of the PhD research

2.1 Background

Continuous application of insecticides has led to the occurrence of resistant malaria vectors (Corbel et al., 2007; Hemingway et al., 2016). Following rigorous distribution of ITNs and application of IRS, malaria vectors have shifted their biting behavior to early evening and outdoor feeding leading to residual malaria transmission and early night biting before people retire to their bed (Killeen et al., 2014). This evolution challenges the current control measures targeting malaria vectors and threatens national malaria control and elimination programs.
Therefore, other malaria vector control tools need to be developed in the fight against malaria in general and more particularly in Ethiopia. One such potential tool is zooprophylaxis (the use of animals to divert blood seeking mosquitoes away from humans) (WHO, 2014). It is previously reported that using animals in close proximity to humans can significantly reduce mosquito biting. However, these tools have not been fully studied due to the success story of LLINs and IRS in vector control. In this study anopheline mosquito behavior and the potential of zooprophylaxis as an alternative strategy for controlling *Anopheles arabiensis*, the major malaria vector in Ethiopia, is investigated.

2.2 Specific objectives

More specifically the objectives of this PhD study are

- To review the role of zooprophylaxis as malaria vector control tool for *Anopheles arabiensis* (Chapter 3),
- To assess the impact of insecticide resistance on malaria vector control interventions (LLINs and IRS) in Ethiopia (Chapter 4),
- To determine host preference of malaria vectors using experimental huts in the study area (Chapter 5) and
- To evaluate the combined effect of repellents and zooprophylaxis in malaria vector control (Chapter 6).

Chapter 3

Zooprophylaxis as malaria control strategy for *Anopheles arabiensis* (Diptera: Culicidae)

Adapted from

Asale, A., Huisman, G., Devleesschauwer, B., Duchateau, L. and Yewhalaw, D. (2016). Zooprophylaxis as malaria control strategy for *Anopheles arabiensis* (Diptera: Culicidae): a systematic review. In preparation.

3.1 Abstract

Zooprophylaxis is the use of wild or domestic animals, which are not the reservoir host of a given disease, to divert the blood-seeking mosquito vectors from human hosts. In this work zooprophylaxis is reviewed systematically to assess its efficacy as a malaria control strategy and to evaluate the possible methods of applying it. The electronic databases PubMed Central, Web of Science and African Journals OnLine were searched using key terms: "zooprophylaxis" or "cattle and malaria" and reports published between January 1995 and March 2016 were incorporated. Thirty-four reports on zooprophylaxis were retained in the systematic review. *Anopheles arabiensis* is an opportunistic feeder. It has a strong preference for cattle odor compared to human odor but feeds on both. Its feeding behavior depends on the available hosts, varying from endophilic and endophagic to exophilic and exophagic. Most research assessed either passive or insecticide zooprophylaxis. Insecticide treatment of cattle proved useful in reducing the human biting rates and malaria incidence. Passive zooprophylaxis can be applied only in malaria vector control if cattle and human dwellings are separated in order to avoid the problem of zoopotentiation. The zooprophylaxis outcome varied per country. It is, therefore, advised to use a site-specific evaluation of its effectiveness in vector control as the behavior of *Anopheles arabiensis* varies per location and circumstances.

Key words: Zooprophylaxis, *Anopheles arabiensis*, malaria, cattle, *Plasmodium* parasites, vector control

3.2 Introduction

Malaria has been known to humans for thousands of years. According to the World Malaria Report 2015 executed by the World Health Organization (WHO), there were an estimated 214 million cases of malaria in 2014, of which approximately 88% were in the African region. Similarly, most of the deaths (90%) also occurred in the African region of which approximately 74% were children under the age of 5. The incidence and death of malaria, however, was reduced by 37% and 60% respectively in 2014 worldwide in comparison to its situation back in 2000 (WHO, 2015).

The malaria parasites are one of the first pathogens to be studied in a public health context due to the high level of morbidity and mortality in humans (Francis, 2010). There are four prominent species of *Plasmodium, P. falciparum, P. vivax, P. malariae* and *P. ovale,* that cause the disease in humans, of which *Plasmodium falciparum* causes most mortality. The different *Plasmodium* species are host specific though there have been periodic reports of simian malaria parasites being found in humans. The disease spreads from one person to another via the bite of female mosquitoes of the genus *Anopheles* (Rich and Xu, 2011). Anopheline mosquitoes belong to the Order Diptera, Family Culicidae and Genus *Anopheles*. There are 476 known *Anopheles* species. Of these only 41 *Anopheles* species are recognized to transmit the *Plasmodium* parasite to humans (Sinka et al., 2012). *Anopheles arabiensis* is one of the member species of the *An. gambiae* complex. The complex also comprises seven additional member species which includes *An. gambiae*, *An. quadriannulatus species* A, *An. amharicus*, *An. melas*, *An. merus*, *An. bwambae* and *An. culuzzi* (Rich and Xu, 2011; Sinka et al., 2012; Yewhalaw et al., 2011; Mathys, 2010; WHO, 1982; Theobald, 1901; Krzywinski and Besansky, 2003; Harbach and Kitching, 1998; Harbach, 2004; Besansky and Fahey, 1997; Sallum et al., 2002). *Anopheles arabiensis* is mainly found in subtropical and tropical savannah regions on the African continent with a majority of its distribution above the equator and along the East coast, including Madagascar, extending farther north into the Sahel, the southwestern corner of the Arabian Peninsula, Kenya, Somalia, south into the desert and steppe environments of Namibia and Botswana in southern Africa (Sinka et al., 2012). The adult *Anopheles arabiensis* is well adapted to dry and forest environments (Afrane et al., 2005; Rúa et al., 2005) whereas the larval habitats are sunlit, clear and shallow water pools (Mereta et al., 2013). The density of larvae increases as the rainy season progresses. The development of the larvae is dependent on the water turbidity and algae (Gimnig et al., 2001; Tuna et al., 2006), thermal limit (Lyons et al., 2012) and maize pollen (Ye-Ebiyo et al., 2000; 2003). It was also suggested that ammonium

sulphate fertilizers increase the larval populations of *Anopheles arabiensis* by decreasing the water turbidity and thereby making it a more attractive breeding site (Mutero et al., 2004; Mwangangi et al., 2006).

In the eastern and southeastern African region where *Anopheles arabiensis* remains the primary vector of malaria, its population dynamics vary according to season with its maximum population density recorded in the long rainy season from June to August (Amel et al., 2002). It survives extreme dry seasons in the form of a dormant embryo in moist soil (Minakawa et al., 2001), continuing reproduction using artificial breeding pans (Musa et al., 2008) and its population quickly builds up the following rainy season as temporary breeding habitats are provided (Amel et al., 2002).

The resting behavior of *Anopheles arabiensis* depends on whether their host resides indoor or outdoor. In areas or at times when the hosts stay mainly indoor, *Anopheles arabiensis* exhibits an endophilic (indoor resting) behavioral pattern (Mnzava et al., 1995). Where hosts are mainly available outdoor, *Anopheles arabiensis* tends to become either resting outdoor (Faye et al., 1997) or indoor (Coluzzi et al., 1979). The exophilic behavior of the female mosquito is also often observed following interventions such as the application of IRS and/or LLINs (Russell et al., 2011; Padonou et al., 2012). A shift from endophilic behavior to exophilic behavior is not only seen in *Anopheles arabiensis* but in all other malaria vector species (Padonou et al., 2012). This shift in mosquito behavior is attributed to the deterrence and/or contact irritancy due to indoor malaria vector control interventions (IRS & LLINs) (Padonou et al., 2012; Reddy et al., 2011; Pates and Curtis, 2005; Mendis et al., 2000).

The most anthropophilic member of the *Anopheles gambiae* complex is *Anopheles gambiae* (Pates et al., 2001b). However, *Anopheles arabiensis* has shown behavioral plasticity in that it exhibits either anthropophagic (Kent et al., 2007) or zoophagic behavior (Chirebvu and Chimbari, 2016). Evaluation of the human blood index of *Anopheles arabiensis* in Ethiopia also showed both zoophagic (Adugna and Petros, 1996; Massebo et al., 2015) and anthropophagic behavior (Tirados et al., 2006).

The zoophilic nature of *Anopheles arabiensis* has been documented in the scientific literature. According to Fornadel et al. (2010), populations of *Anopheles arabiensis* from Zambia showed an anthropophilic behavioral pattern. Other reports from southern Ethiopia indicated that *Anopheles arabiensis* is inherently anthropophilic although it takes relatively high proportions of blood meals from non-human hosts (Tirados et al., 2006). Similarly, in blood meal analysis of populations of *Anopheles gambiae* and *Anopheles arabiensis* from Senegal preference for humans compared to other non-vertebrate hosts was observed (Fontenille, et al., 1997). In

contrast, a study from Ethiopia showed a smaller proportion of human blood taken from areas with mixed dwellings (Hadis et al., 1997). Exclusive zoophilic behavior of *Anopheles arabiensis* was reported from Madagascar (Duchemin et al., 2001). Other studies on populations of *Anopheles arabiensis* from other countries, however, showed an opportunistic feeding behavior (Gillies and Coetzee, 1987).

The time of host-feeding varies depending on the host preference and on indoor or outdoor availability of the host. In an assessment of the hourly man-biting rates of *Anopheles gambiae s.l.* in Miwani, Kenya, a region where *Anopheles gambiae* (54%) and *Anopheles arabiensis* (45%) exist in sympatry, the majority (83%) of the female mosquitoes were found biting between 01:00 and 06:00 h, with a peak indoor biting at 06:00 h while, the peak outdoor activity occurred between 02:00 and 04:00 h (Githeko et al., 1996a). In Ahero village, where *Anopheles funestus* comprised a large proportion of mosquitoes caught indoor (67.3%), the main indoor biting peak for *Anopheles arabiensis* occurred at 03:00 h while the outdoor biting activity peaked between 03:00 and 06:00 h. The same study concluded that *Anopheles arabiensis* was 1.9 times more likely to bite indoor than outdoor and that this mosquito had very low preference for human blood meals as compared to *Anopheles gambiae.* However, Taye et al. (2006) reported that *Anopheles arabiensis* in southern Ethiopia bites during the entire night with a peak between 23:00 h and 03:00 h. A recent study by Yohannes and Boelee (2012) from northern Ethiopia showed that *Anopheles arabiensis* has more early biting activities with 70% of the biting activity occurring before 22:00 h, with a peak between 19:00 h and 20:00 h which is similar with the report from Kibret et al. (2010) from central Ethiopia.

A difference in the time of biting and rhythm seems to be affected by parity, with a larger proportion of possibly disease transmitting parous mosquitoes being active in the latter part of the night, mainly when humans sleep (Taye et al., 2006; Robert and Carnevale, 1991). Seasonality can also influence the biting activity of populations of *Anopheles arabiensis.* Taye et al. (2006) documented that the biting rate of *Anopheles arabiensis* in August and April was 19.3 bites/person/night and 82 bites/person/night, respectively.

With regard to the biting place on the human body, *Anopheles* mosquitoes often portray a preference for a specific body part, seldom displaying a random biting pattern on theirs hosts. *Anopheles arabiensis* as well as *Anopheles gambiae* have a strong preference for the legs, feet and ankles (Govere et al., 2001; Karunamoorthi et al., 2010). Important malaria vectors are unequally distributed within a country with their range typically crossing national borders. The occurrence of *Anopheles* species varies according to macro- and micro-environmental differences exhibited by different bio-ecological areas. Most entomological studies should

incorporate a detailed distribution of the vector mosquito species, as it is an important factor in the risk assessment of malaria transmission (Costantini et al., 2009; Kashiwada and Ohta, 2010). Thus, the abundance of anophelines is one of the key entomological parameters used to describe the relationship between vectors and the incidence of malaria (Galardo et al., 2009).

One of the keystones in malaria control strategy is tackling the vector, either by reducing the vector density or infectivity rate of the vector which will have an impact on malaria transmission and incidence. Based on previous research reports, it appears that the mosquito population has developed resistance against most insecticides (DDT, permethrin, deltamethrin and malathion) (Yewhalaw et al., 2011). Despite the success of existing vector control intervention strategies such as long lasting insecticidal treated nets (LLINs) and indoor residual spray (IRS), the emergence and spread of insecticide resistance in some regions suggest that other vector-control tools may be needed to sustain control and mitigate the risk of malaria infection (Yewhalaw et al., 2011). Consequently, new attention has been given to environmental management, biological control and zooprophylaxis (Mathys, 2010).

In malaria vector control, zooprophylaxis can be applied separately or in combination with other vector control tools in some instances. Application of zooprophylaxis is the use of wild or domestic animals, which are not the reservoir host of a given disease, to divert the blood-seeking mosquito vectors away from the human host of that disease. Use of zooprophylaxis as a malaria vector control tool can be in an active, passive or integrated form combined with chemical insecticides used in public health (Mathys, 2010; WHO, 1981). Research assessing the effectiveness of zooprophylaxis has been done in various countries. In this paper the role of zooprophylaxis as malaria vector control tool is reviewed.

3.3 Methods

3.3.1 Identification of papers and selection criteria

The data bases PubMed Central, Web of Science, Science direct and African Journals OnLine were searched and reports published between January 1995 and March 2016 were incorporated. The search was limited to abstracts and full texts in English. The published reports used in this review were retrieved from database searches for key terms: “zooprophylaxis”, “cattle and malaria”, “malaria vector control” or “host preference”. In cases where key terms could not produce enough relevant information, references from related articles were copied and pasted in google scholar to get the full PDF of the target article. Review articles on zooprophylaxis

were excluded from the synthesis but their content was assessed in order to evaluate their objective, their relevance and relatedness to our review and their inclusiveness of contemporary information. Abstracts were selected if they were found to include information on zooprophylaxis, malaria control strategies or on the behavior of malaria vectors and their host preference. Irretrievable full text articles as well as non-English abstracts were excluded during the selection.

The selected articles were screened as follows. First all abstracts not related to *Anopheles* resting, feeding behavior, feeding pattern, host preference, zooprophylaxis, or the diversion of mosquitoes to hosts other than humans were excluded. Second duplicate and non-malaria related articles were not considered in the review. Bulletin news and articles reviewing the effects of zooprophylaxis discussed in other reviews were also excluded from the selection (Figure 3.1). Data extraction from each article included author, date of publication, study location, mosquito species, study aim, study design and study outcomes. Published research works reporting significant association between presence of livestock and reduced malaria infection were considered as supporting the use of zooprophylaxis and studies that either report failure of zooprophylaxis or poor association of zooprophylaxis and reduced malaria infection were considered to disprove the use of zooprophylaxis in malaria vector control. The review article first provides a brief description of *Anopheles* behavior with priority given to *Anopheles arabiensis*. Then research results on zooprophylaxis are outlined and discussed and conclusions are drawn with respect to the use of zooprophylaxis as a vector control tool.

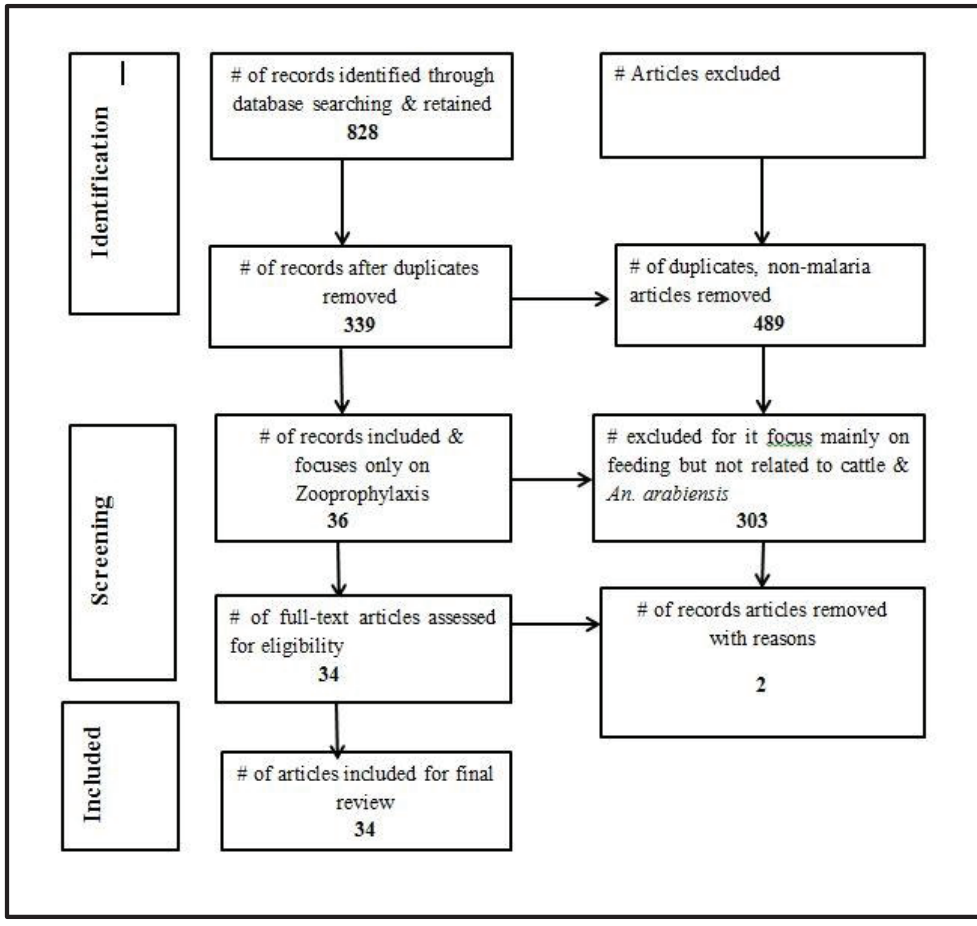

Figure 3.1 Flow chart for systematic article selection

3.4 Results

3.4.1 Description of study characteristics

Thirty-four articles were included in this review for the role of zooprophylaxis in malaria vector control (Figure 3.1). Of the total 34 selected articles, 13 research articles (38%) showed that zooprophylaxis was effective in malaria vector control. Of these 13 supporting articles, 4 research works were conducted in Asia (India, Indonesia and Pakistan), whereas the remaining 9 were reported from Africa (1 West Africa, and 8 East Africa). Concerning the study design, 2 were case-control, 2 were cross sectional, 1 was a randomized controlled trial and 8 were experimental studies. Another thirteen research articles (38%) were found to show that zooprophylaxis increased the incidence of malaria, or showed no effect at all on malaria infection. About their study design, 3 were field studies, 2 were paired cohort studies, 2 were case control studies and the remainder 6 were cross sectional surveys. The last 8 articles (24%) are modeling studies reporting the role of zooprophylaxis in malaria vector control.

3.4.2 Outcome parameter measured

Ten studies measured parasitaemia and/or vector abundance, 11 studies measured mosquito abundance, human blood index (HBI) and/or sporozoite rate, 4 studies measured mosquito mortality and knockdown, 2 studies mosquito biting behavior and human landing catch (HLC) and finally one study used physiological status and mosquito mortality.

3.4.3 The role of zooprophylaxis in malaria control

The role of domestic animals, particularly cattle, in reducing malaria incidence differs with the zooprophylaxis type, which can be categorized as passive, active, combination and insecticide zooprophylaxis. Passive zooprophylaxis is the natural prophylactic effect of cattle that is seen when cattle density within a community is increased. Its effect can be studied by evaluating the association between domestic animal ownership and parasitaemia (Bulterys et al., 2009; Iwashita et al., 2014; Yamamoto et al., 2009), or mosquito blood meal source, mosquito infectivity (Habtewold et al., 2001; Iwashita et al., 2014; Kaburi et al., 2009; Tirados et al., 2006), or mosquito density (Hadis et al., 1997; Muriu et al., 2008; Mahande et al., 2007a).
Active zooprophylaxis on the other hand refers to the deliberate introduction of domestic animals in order to divert mosquitoes away from human settlements towards other non-transmitting hosts. Active zooprophylaxis is studied by evaluating the association between

malaria prevalence and cattle ownership using paired cohort studies of people sleeping with cattle placed at close proximity and people sleeping with cattle placed at a distance (Bogh et al., 2001; 2002).

In combination zooprophylaxis, zooprophylaxis is combined with insecticide treated nets (ITN) and IRS in order to induce a push-pull effect, thereby aiming at a reduced risk of disease incidence. The deliberate introduction of long lasting insecticide treated nets (LLIN) and IRS is considered as the pushing factor whereas domestic animals are used as the pulling factor. The effect is studied by evaluating the association between ITN ownership, IRS coverage, livestock ownership and malaria prevalence (Iwashita et al., 2014; Kaburi et al., 2009)

Insecticide zooprophylaxis is the treatment of cattle by sponging or dipping with insecticides in order to pass on a lethal dose of insecticides to the blood-feeding mosquitoes. This effect can be studied by evaluating the difference in mosquito mortality and density, and malaria incidence in households that possess treated domestic animals and untreated domestic animals (Lyimo et al., 2012; Fritz et al., 2009; Mahande et al., 2007a; Rowland et al., 2001; Foley et al., 2000; Hewitt and Rowland, 1999; Habtewold et al., 2004).

Pig and donkey keeping is reported to be a risk factor for malaria transmission in Mozambique (Temu et al., 2012), Guinea Bissau (Palsson et al., 2004) and Burkina Faso (Yamamoto et al., 2009). Similarly, Bouma and Rowland (1995) noticed an increased *Plasmodium* prevalence in children in Pakistan living in households with cattle and Githinji et al. (2009) concluded that the presence of cattle and long grass in the homesteads result in a 1.81 higher risk for malaria infection in Kenya. Research in the Gambia by Bogh et al. (2001; 2002) suggested that passive zooprophylaxis was effective. The decrease in parasitaemia, however, was attributed to the fact that cattle owners were wealthier than non-cattle owners. Tirados et al. (2006) conducted an entomological study on *Anopheles arabiensis* and *Anopheles pharoensis* in Arba Minch, southwestern Ethiopia in order to determine the host preference, resting behavior of vector population and protective value of cattle against malaria. They concluded that cattle have protective value against *Anopheles pharoensis* both indoor and outdoor. *Anopheles arabiensis* from this area remains anthropophagic, exophagic and exophilic and can sufficiently feed on human to transmit the disease. Therefore, humans staying indoor are only mildly protected if cattle are placed outdoor. Habtewold et al. (2004) also assessed the effectiveness of deltamethrin-treated zebu and the related behavioral avoidance of *Anopheles arabiensis* in the same region and concluded that cattle have a protective value against *Anopheles pharoensis.* However, no zooprophylactic effect was observed by placing zebu cattle near humans for *Anopheles arabiensis*. Similarly, in studying the risk factors associated with malaria incidence,

it was concluded that humans sleeping in the house with animals have a significantly higher risk of malaria both in Ethiopia (Derressa et al., 2007; Ghebreyesus et al., 2000) and Pakistan (Idrees and Jan, 2001).

A number of reports and modeling studies argue that zooprophylaxis is effective under specific circumstances. According to Tirados et al. (2011), zooprophylaxis is only effective for *Anopheles arabiensis* when humans rest indoors and cattle remain outdoors. Human biting rate was reported to be highest in mixed dwellings and lowest when cattle are kept separately both in Ethiopia (Seyoum et al., 2002) and Zambia (Bulterys et al., 2009). This is also supported by modeling studies by Hassanali et al. (2008), Kawaguchi et al. (2004) and Saul (2003) who argued that separating the habitats of cattle and humans is necessary for the success of zooprophylaxis. This is due to the fact that the presence of cattle may decrease malaria transmission to humans but increase mosquito survival rate. In addition to habitat separation the animal population should increase above a threshold value, causing the diversion of the mosquitoes to be a more effective malaria control strategy than decreasing the mosquito population (Franco et al., 2014; Nah et al., 2010). Reports confirming the effectiveness of zooprophylaxis were made in both African and Asian countries. Of these reports, 6 studies were field experiments on insecticide zooprophylaxis. The successfully used treatments on cattle included a fungus (bio-insecticide zooprophylaxis) (Lyimo et al., 2012), ivermectin (Fritz et al., 2009; Foley et al., 2000), deltamethrin, (Mahande et al., 2007a; Rowland et al., 2001; Hewitt and Rowland, 1999), permethrin and lambdacyhalomethrin (Hewitt and Rowland, 1999). Fungal, ivermectin and deltamethrin-treated animals significantly reduced survival rates of malaria vectors as well as fecundity. Residual effects were longest in deltamethrin-treated cattle. Studies on passive zooprophylaxis consisted mainly of population-based case control studies and surveys. In these studies, different household risks for the transmission of malaria were evaluated. The combination effect of ITN, IRS and livestock was also assessed (Kaburi et al., 2009; Iwashita et al., 2014; Levens, 2013; Killeen and Smith, 2007; Kawaguchi et al., 2004).

Mahande et al. (2007a) investigated feeding and resting habit of *Anopheles arabiensis* using indoor and outdoor collections. They compared mosquito density attracted to different odor sources including cattle, sheep, goat and human. They also assessed HBI of mosquitoes collected from both indoor and outdoor sources. They observed a decrease in HBI and protective value of cattle against *Anopheles arabiensis*. Similarly, Habtewold et al. (2001) investigated mosquito density, source of blood meal and mosquito infectivity rate in the presence of cattle and observed a decrease in HBI and protective value of cattle and goat.

Deressa et al. (2007), Kaburi et al. (2009) and Iwashita et al. (2014) collected mosquitoes from households, made inventories of livestock and assessed the presence or absence of ITN per household in Kenya. They showed that both the man-biting rate as well as the HBI of *Anopheles arabiensis* decreased with increase of the number of cattle in households with ITN, demonstrating the additive role of livestock and ITN. This is also supported by modeling studies by Levens (2013) and Killeen and Smith (2007) who argued that mass coverage of LLIN up to 80% to the community and 80% livestock treatment with pyrethroids could lead to a global reduction and elimination of the disease.

The separation of human shelters and animal sheds at a certain distance (Iwashita et al., 2014; Bogh et al., 2001; 2002; Ghebreyesus et al., 2000; Bouma and Rowland, 1995) can be combined with the use of LLIN and IRS (Iwashita et al., 2014; Kaburi et al., 2009) and the treatment of domestic animals with appropriate insecticides (Lyimo et al., 2012; Fritz et al., 2009; Mahande et al., 2007a; Rowland et al., 2001; Foley et al., 2000; Hewitt and Rowland, 1999; Habtewold et al., 2004). The efficacy of zooprophylaxis is affected by the type of mosquito species and its feeding and resting behavior. Thus, ownership of domestic animals in the presence of anthropophilic vectors such as *Anopheles gambiae* and *Anopheles funestus* may lead to increased risk of malaria incidence. In contrast, ownership of domestic animals may lead to lower risk of malaria incidence in areas where zoophilic and/or opportunistic vector species such as *Anopheles arabiensis* and *Anopheles pharoensis* predominate (Bogh et al., 2002; Habtewold et al., 2004; Iwashita et al., 2014; Tirados et al., 2006). Studies related to the efficacy of zooprophylaxis are presented in table 3.1, 3.2 and 3.3.

Table 3.1 Summary of methodological aspects of reviewed publications

Reference	Location	Study aim	Study design	Sample size
Lyimo et al., 2012	Kilombero, Tanzania	Evaluating the effectiveness of fungus bio-insecticide zooprophylaxis	Semi-field and small-scale field experiment	1,690 from Semi-field and 547 *An. arabiensis* from field assessed for development of fungal infection
Kaburi et all., 2009	Kenya	Establishing effects of zooprophylaxis and LLNs	Cross-sectional survey	80 households surveyed. 4,148 and 2,615 vector mosquitoes were collected before and after intervention respectively and blood source detected.
Bulterys et al., 2009	Zambia	Association between malaria infection & risk factors	Case-control studies	34 households with malaria history in the last 2 years & 37 households without malaria history in the same time period were assessed for risk factors
Fritz et al., 2009	Kenya	Effects of ivermectin and moxidectine on malaria vectors	Laboratory-based & field-based bio-assays	Exact sample size not mentioned
Muriu et al., 2008	Kenya	To determine the blood feeding pattern of *Anopheles* mosquitoes	Field study	3,333, blood fed Anopheles mosquitoes were collected from 8 villages and blood source detected.
Mahande et al.,2007a	Tanzania	Evaluation of the feeding preference behavior	Field experiment	3,902 Anopheles collected from field and blood source detected, 506 Anopheles were trapped using OBET and preference detected
Mahande et al., 2007b	Tanzania	Assessing the effect of deltamethrin-treated cattle on *An. Arabiensis*	Contact Bioassay & Experimental hut trials	948 female *An. arabiensis* were used for contact bioassay
Iwashita et al., (2014)	Kenya	Assessing the added value of zooprophylaxis in the presence of ITN	Cross sectional study	1664 Anopheles mosquitoes were examined for blood meal source & vector infection rate

Table 3.1 continued

Reference	Location	Study aim	Study design	Sample size
Seyoum et al., 2002	Ethiopia	To assess the impact of livestock on the human biting rate & malaria transmission	Human landing catch(HLC), and parasitological survey	Mosquitoes were collected using HLC for 12 months(once/month/3huts) and 1,180 blood samples were collected from children under age 10
Habtewold et al., 2001	Ethiopia	A blood meal analysis to determine the host preference	Field experiment	278 mosquitoes were tested for the blood meals source and parasite positivity
Rowland et al., 2001	Pakistan	The role of insecticide treated livestock (dipping method) in control of malaria	Randomized controlled trial	842 Anopheles mosquitoes were monitored, an average 4,112 blood samples were collected and tested for parasite detection over 3-year period.
Foley et al., (2000)	Indonesia	The effect of ivermectin-treated animals and humans on *An. farauti* mortality	Experimental trial and modeling	Exact sample size not reported
Hewitt and Rowland (1999)	Pakistan	The treatment of cattle with pyrethroids to control zoophilic mosquitoes	Field experimental trial	Over 38,815 Anopheline mosquitoes were collected over 2-year period.
Temu et al., 2012	Mozambique	Identifying risk factors for malaria infections	Cross-sectional survey	8,338 children under 15-years were screened for malaria detection
Tirados et al., 2011	Ethiopia	Attraction of mosquitoes to humans in the absence and presence of cattle	Field study	Exact sample size not mentioned
Yamamoto et al., 2009	Burkina Faso	The use, effects of different mosquito control measures	Case-control study	117 cases and 221 control study subjects were screened for parasite
Githinji et al., 2009	Kenya	Interactions between humans & their micro-ecological environment	Case-control studies	342 case and 328 control individuals were assessed risk factors associated to malaria
Deressa et al., (2007)	Ethiopia	Household & socio-economic factors associated with childhood febrile illness	Cross-sectional survey	2,372 households were investigated for risk factors associated with malaria.

Table 3.1 continued

Reference	Location	Study aim	Study design	Sample size
Tirados et al., 2006	Ethiopia	Feeding & resting preference, to evaluate the protective value of cattle against *An. arabiensis*	Field experimental study	More than 45, 000 *An. arabiensis* mosquitoes were collected
Palsson et al., 2004	Guinea Bissau	Environmental risk factors associated with increased malaria risk and vector abundance	Cross-sectional survey	9,873 Anopheles mosquitoes were collected over 3-year period.
Habtewold et al., (2004)	Ethiopia	Deltamethrin-treated Zebu & possible behavioral avoidance of *An. Arabiensis*	Field experiments	Over 1,084 Anopheles mosquitoes were monitored for feeding success. 366 Anopheles mosquitoes were tested for blood meal source.
Bøgh et al., (2002)	The Gambia	Effect of passive zooprophylaxis on malaria transmission	Paired cohort study of 102	A total of 204 children were monitored for malaria in the presence and absence of cattle
Bøgh et al., (2001)	The Gambia	Effect of passive zooprophylaxis on malaria transmission	A paired cohort of 102 children study	A total of 204 children were monitored for malaria in the presence and absence of cattle Mosquito density was also monitored
Idrees & Jan, (2001)	Pakistan	To determine the role of cattle ownership on the prevalence of malaria	parasite prevalence survey	1,873 blood samples were collected and tested for malaria.
Ghebreyesus et al.,(2000)	Ethiopia	Household risk factors associated with malaria incidence	Cross-sectional survey	2,114 children under 10 were screened for malaria and risk factors.
Bouma & Rowland (1995)	Pakistan	Parasite prevalence in children housing with or without cattle	Parasitological survey	2,042 blood samples were collected from school children under age 15 over 2 years

Mayagaya et al., 2015	Tanzania	To investigate the impact livestock ownership on vector ecology and malaria parasite infectivity rate	Field mosquito collection	29,393 Anopheles mosquitoes were collected over 3-year period.

Table 3.1 Summary of major findings of reviewed publications

Reference	Mosquito species	Outcome parameter	Percent protection	Conclusion (Yes/no) to zooprophylaxis
Lyimo et al., 2012	*An. Arabiensis*	Mosquito mortality Fecundity	90% of mosquitoes fed on fungus treated cattle become infected immediately & 70% infection after 3 days	Yes (if cattle treated with bio-insecticide)
Kaburi et all., 2009	*An. gambiae* complex and *An. Funestus*	Man biting rate (MBR) Human blood index (HBI) Circumsporozoite test (CSP)	MBR ratio decreased significantly, with RC of (–0.96; SE = 0.834; p = 0.017. HBI decreased significantly with RC of (0.239; SE = 0.039; p = 0.015 (p <0.05) especially in households with >4 cattle	Yes (if cattle and LLINs co-applied)
Bulterys et al., 2009	*An. arabiensis* and *An. Funestes*	Parasite prevalence	Reduced the risk of *P. falciparum* infection (OR = 0.13; 95% CI = 0.03–0.56).	Yes (if cattle sheds are separated from human quarters)
Fritz et al., 2009	*An. gambiae* s.s. and *An. Arabiensis*	Mosquito density Human blood index	90% mortality of mosquitoes fed on ivermectin treated cattle	Yes (if cattle treated with systemic insecticide)
Muriu et al., 2008	*An.arabiensis An. pharoensis An. funestus*	Human blood index Bovine blood index(BBI)	71.8% indoor and 41.3% outdoor collected mosquitoes were fed on bovine.	Yes
Mahande et al.,2007a	*An. arabiensis* and *An. Gambiae*	Mosquito density HBI	90.3% of mosquitoes were trapped by cattle odor & 9.7% of mosquitoes were trapped by human	Yes (if cattle kept in human surroundings)

			odor (P=0.005). lower HBI was recorded in both outdoors (0.1–0.3) & indoor (0.4–0.9) collected mosquitoes .	

Table 3.2 continued

Reference	Mosquito species	Outcome parameter	Percent protection	Conclusion (Yes/no) to zooprophylaxis
Mahande et al., 2007b	*An. Arabiensis*	Mosquito mortality, HBI,	50% of mosquitoes fed on treated cattle were knocked down 21 days of treatment Treated cows caused higher mortality (mean=2) as compared to untreated (m=0.3)	Yes (if cattle is treated with deltamethrin every 3 week)
Iwashita et al., (2014)	*An. arabiensis* *An. gambiae* s.s *An. funestus* s.s	Mosquito density Circumsporozoite test (CSP) rate	40.5% (CI: 36.9-44.2) of *An. arabiensis* fed on cattle. 12.0% (CI: 9.7-14.6) of *An. arabiensis* fed on humans. ITN and cattle are associate with decreased CSP	Yes (if cattle co-applied with ITN)
Seyoum et al., 2002	*An. arabiensis,* *An. Pharoensis*	Human biting rate (HBR) Parasite prevalence	HBR of *An . arabiensis* in mixed, separate cattle and without cattle were 8.45, 4.64 & 5.97 respectively. Similarly mean parasitaemia was 26.7%, 15.0% & 23.85% respectively	Yes (if cattle is separated from human dwelling)
Habtewold et al., 2001	*An. arabiensis, An.quadriannulatus*	Mosquito density HBI,	Significantly higher proportion of mosquitoes were fed on live stock in site C compared to site A (x^2=44.1, Df=1, p<0.001) or B (x^2=25.9,Df=1, P<0001)	Yes (in certain areas)
Rowland et al., 2001	*An. stephensi , An. Culicifacies*	Mosquito mortality, Parasite prevalence	56% reduction in *Pf.,* 31% reduction *pv.*	Yes (If cattle treated with insecticide)
Foley et al., (2000)	*An. Farauti*	Mosquito mortality	80-100% mortality of mosquitoes fed on treated cattle in the first 3 days after treatment	Yes (If cattle treated with insecticide)
Hewitt and Rowland (1999)	*An. stephensi, An. Culifacies*	Mosquito mortality	50% reduction in longest vector survivors	Yes (If cattle treated with insecticide)

Table 3.3 continued

Reference	Mosquito species	Outcome parameter	Percent protection	Conclusion (Yes/no) to zooprophylaxis
Temu et al., 2012	*An. gambiae* complex, *An. Funestus*	Malaria incidence	Increased risk of malaria incidence (OR = 3.2; 95%CI: 2.1-4.9).	No
Tirados et al., 2011	*An. arabiensis* and *An. Pharoensis*	Mosquito density	50% decrease in *An. Pharoensis* No sig. difference in *An. arabiensis*	No
Yamamoto et al., 2009	*An. gambiae* s.l. & *An. Funestus*	Mosquito Density, Parasite prevalence	Positively correlation between donkeys & *An. gambiae* indoors (Pearson's r = 0.21, P = 0.0002).	No
Githinji et al., 2009	*An. gambiae* s.l.	parasitaemia	53% increased risk of acquiring malaria if oxen kept in the house	NO
Deressa et al., (2007)	-	Parasitaemia	Sharing house with livestock increases the risk of malaria(OR = 1.3, 95%CI 1.1-1.6)	No
Tirados et al., 2006	*An. arabiensis* and *An. pharoensis*	Mosquito density HBI CSP	HBI for Outdoor & indoor recorded 51% and 66% respectively. CSP for *Pf.* & *Pv*. Recorded 0.3% & 0.5% respectively. 5x more mosquitoes inside human baited trap	No
Habtewold et al., (2004)	*An. arabiensis* *An. pharoensis* *An. tenebrosus*	Mosquito mortality	Zebu cattle treated with deltamethrin provoked 50% mortality in mosquitoes up on feeding after 4th week.	No (reduction in density was not significant)
Bøgh et al., (2002)	*An. gambiae, An. arabiensis, An. Melas*	Parasitaemia	No significant differences in either the risk of parasitaemia (OR = 1.69, P = 0.26) or in high-density parasitaemia (OR = 0.73, P = 0.54).	No

Table 3.2 continued

Reference	Mosquito species	Outcome parameter	Percent protection	Conclusion (Yes/no) to zooprophylaxis
Bøgh et al., (2001)	*An. gambiae, An. arabiensis, An. melas*	Parasitaemia HBI CSP	There was no significant difference between case and control groups for all parameters measured.	NO
Idrees & Jan, (2001)		Parasitaemia	11% parasite rate in children of families which kept cattle inside as compared to 7.1% who did not keep.	No
Ghebreyesus et al.,(2000)		Parasitaemia	Human sleeping in the houses with animals were significantly associated with risk of malaria (RR = 1.92, 95% CI 1.29-2.85).	no
Bouma & Rowland (1995)		Parasitaemia	Higher mean prevalence of *Plasmodium* infection in children housing with cattle (15.2% versus 9.5%; P<0.005) without.	No
Mayagaya et al., 2015	*An. arabiensis* *An. Funestus*	Mosquito density HBI	Livestock ownership significantly alters vector resting and feeding behavior. There was significantly reduced HBI in houses livestock, however, its implication in reduced malaria transmission is not clear.	No

Table 3.4 Summary of publications showing different models on introduction of zooprophylaxis as malaria vector control tool

Authors	Species	Study aim	Study design	Recommendations on use of Zooprophylaxis
Franco et al., 2014	*An. stephensi, An. Arabiensis*	To model the role of livestock in malaria control	Mathematical model	Livestock could have zooprophylactic effect with certain conditions such as maximum density of vector population prior to introduction, sufficiently high no of livestock. Treatment of livestock with non-repellent insecticides & increasing the attractiveness of livestock with attractants will maximize the efficacy.
Levens (2013)	*An. Arabiensis*	To model the role of insecticide zooprophylaxis, LLIN	Mathematical model	More than 80% coverage of LLIN to community and 80% coverage of insecticide treatment to the livestock are important to achieve global reduction and elimination of the disease.
Nah et al., (2010)	*An. Sinensis*	To investigate he effect of zooprophylaxis	Mathematical model	Decrease of animal population increases the basic reproduction number R0. Passive zooprophylaxis is effective malaria control strategy in South Korea.
Hassanali et al., 2008		Relationship between hosts, mosquito habitat & the relative no. of individuals in the group	Computer simulation	When the distance between human & animal host increases the no. of bites/person first decrease & then followed by an increase in the number of bites. Animals should be placed in certain optimum place.
Killeen & Smith (2007)	*An. Arabiensis An. Gambiae*	To predict the effect of mass coverage of LLIN on users and non-users	Computer simulation	Mass coverage of LLIN & IRS capable of excito-repellency in the presence of cattle it is possible to protect both the users & non-users of ITN.
Kawaguchi et al., (2004)		Combining zooprophylaxis & Indoor residual spraying	A simulation model	Habitat separation of cattle and humans is important for the success of zooprophylaxis. When blood host density is below the blood feeding satiation level, zooprophylaxis will fail. Spraying insecticides at human dwellings diverts mosquitoes to other hosts.

Table 3.3 continued

Authors	Species	Study aim	Study design	Recommendations on use of Zooprophylaxis
Saul (2003)	NA	Examining the effects of animals on the transmission of vector-borne diseases	Computer simulation	Feeding on animal's decreases transmission to humans but increases mosquito survival rate. Keeping animals & humans away from breeding sites is a practical control measure. Insecticide zooprophylaxis may reduce vectorial capacity.
Killeen et al., (2001)	*An. funestes,* *An.gambiae* *An. arabiensis*	The influence of host availability on vector blood meal choice	Computer Simulation model	Increased cattle populations would cause a significant reduction in malaria in The Gambia due to a high *An. arabiensis* population, compared to no significant influence in Tanzania.

3.5 Discussion

Malaria remains a major burden in Sub-Saharan Africa and continually finding effective control strategies is of great importance. Before zooprophylaxis can be used as a control strategy, several conditions are required. A zoophilic and exophilic vector is the most essential component for zooprophylaxis to be effective. Then habitat separation between human and host animal quarters is the second most important condition. Third zooprophylaxis can be augmented further through insecticide treatment of the animal, co-intervention of LLINs or/and IRS.

The main zoophilic vectors identified with successful zooprophylaxis were *An. arabiensis, An. pharoensis* in Africa (Kaburi et al., 2009; Bulterys et al., 2009; Mahande et al., 2007a; Seyoum et al., 2002; Habtewold et al., 2001; Tirados et al., 2011) and *An. stephensi, An. culifacies, An. sinensis* and *An. farauti* in Asia (Rowland et al., 2001; Foley et al., 2000; Hewitt and Rowland, 1999; Nah et al., 2010). *An. arabiensis* is one of the main vectors of malaria in Sub-Saharan Africa. It is known mostly for zoophilic (Duchemin et al., 2001; Habtewold et al., 2001; Mahande et al., 2007; Kaburi et al., 2009; Massebo et al., 2015), opportunistic (Animut et al., 2013; Haddis et al., 1997) and occasionally anthropophilic (Tirados et al., 2006; Fornadel et al., 2010; Kent et al., 2007) behavior. Thus the behavior of *An. arabiensis* can be varied depending on the location of the host (indoor vs outdoor) and local genotype of vector population with the west African population mostly identified as anthropophilic and the eastern counterpart being more zoophilic (Bogh et al., 2001; Tirados et al., 2006). It may therefore be concluded that *Anopheles arabiensis* is an opportunistic feeder, feeding on both human and cattle depending on host availability. This is the basis of a line of thought that zooprophylaxis can be introduced to control malaria where *An. arabiensis* is the main malaria vector,

Separation of human living house and livestock quarters was found to be another key precondition in the process of implementing zooprophylaxis. This was evidenced when in almost all instances where people and livestock shared the same house, ended up in higher risk of malaria infection (Temu et al., 2012; Palsson et al., 2004; Yamamoto et al., 2009; Bouma and Rowland, 1995; Githinji et al. (2009). Thus, the presence of cattle may reduce the HBR as well as the HBI but that is no guarantee for decreasing the estimated transmission risk or having a significant prophylactic effect. The fact that cattle may play a role as attractant for vectors to human resting places has been proven in several reports (Temu et al., 2012; Tirados et al., 2011; Yamamoto et al., 2009; Githinji et al., 2009; Deressa et al., 2007; Palsson et al., 2004;

Idrees and Jan, 2001; Ghebreyesus et al., 2000; Bouma and Rowland, 1995; Mayagaya et al., 2015).

In addition to the presence of a zoophilic vector and the separation of human living house, zooprophylaxis can be further strengthened if augmented with other interventions. This may include treatment of livestock with insecticides with the primary purpose of toxicating mosquitoes fed on the animal. With regard to this there are successful reports including fungus formulations (bio-insecticide zooprophylaxis) (Lyimo et al., 2012), ivermectin (Fritz et al., 2009; Foley et al., 2000), deltamethrin, (Mahande et al., 2007a; Rowland et al., 2001; Hewitt and Rowland, 1999), permethrin and lambdacyhalomethrin (Hewitt and Rowland, 1999). In all instances, insecticide treated animals significantly reduced survival rates of malaria vectors as well as fecundity. Residual effects were longest in deltamethrin-treated cattle. Furthermore, lower risk of malaria was reported when zooprophylaxis and other main vector tools (LLINs and IRS) are used in combination (Kaburi et al., 2009; Iwashita et al., 2014; Levens, 2013; Killeen and Smith, 2007; Kawaguchi et al., 2004).

As a negative side effect, the presence of cattle may lead to a higher survival rate of *Anopheles arabiensis* due to the abundance of available blood meals, increasing the mosquito population. This phenomenon of zoopotentiation calls for the need to evaluate zooprophylaxis as a control strategy thoroughly before introducing it into a community. Zoopotentiation may not only occur through an increase in blood meals and host availability, but cattle puddles provide an ideal breeding site for the development of mosquito larvae, hence increasing the mosquito population (Saul, 2003; Killeen et al., 2001).

Another point of caution is the fact that when mosquito abundance is enlarged, other vector-borne diseases of humans or animals may increase in incidence. When viewing the various kinds of zooprophylaxis, both passive and active zooprophylaxis only divert mosquitoes to different hosts but cause no decrease in vector abundance. The advantage of insecticide zooprophylaxis is the ability to reduce the survival and fecundity of the mosquito. However, reducing the survival rate and fecundity of the mosquitoes is not necessarily beneficial. A decrease in the number of zoophilic vectors may give rise to an increase of a different and possibly more anthropophilic vector indirectly via decreased competition for larval space and resource. The result would be that insecticide zooprophylaxis would only reduce malaria transmission temporarily. Thus, further research on the possible consequences of the use of insecticide zooprophylaxis is required to make a more accurate evaluation.

3.6 Conclusion

In conclusion, zooprophylaxis should be evaluated in a site-specific approach, as it has been reported to be effective in some regions and in others not. The effectiveness depends on several factors including housing, the distance to the breeding site of mosquitoes and the use of other control strategies such as ITNs and IRS. These factors influence the resting behavior of the local malaria mosquitoes. Moreover, the zoophilic behavior of *Anopheles arabiensis* varies in the different African countries, showing a more anthropophilic behavior in West Africa compared to countries lying more East on the continent. This would suggest that zooprophylaxis could be more effective in East African countries, especially in Madagascar where the species is said to be fully zoophilic. The use of other malaria control strategies may also have influenced the evaluated results of experiments on zooprophylaxis.

Exclusions and abstract selections were made by one person. A more objective selection of reports may also be made by letting a number of people independently chose whether or not to include or exclude certain reports. This could result in a more detailed description of the different methods used in experiments on zooprophylaxis. Future studies such as estimation of the distance threshold between human quarters and livestock pen, the additive effect of repellent and zooprophylaxis could further strengthen the value of zooprophylaxis.

3.7 References

Adugna, N. and Petros, B. (1996). Determination of the human blood index of some anopheline mosquitos by using ELISA. *Eth. Med. J.* 34, 1-10.

Afrane, Y.A., Lawson, B.W., Githeko, A.K. and Yan, G. (2005). Effects of microclimatic changes caused by land use and land cover on duration of gonotrophic cycles of *Anopheles gambiae* (*Diptera: Culicidae*) in western Kenya highlands. *J. Med. Entomol.* 42, 974-980.

Amel, A.H., Abd el Hamid, D.N., David, E. A., Haider, A.G., Abdel-Musin, A.A., Gwiria, M.S., Theander, T.G., Alison, M. C., Hamza, A.B. and Dia-Eldin, A.E. (2002). A marked seasonality of malaria transmission in two rural sites in eastern Sudan. *Acta Trop.* 83, 71-82.

Besansky, N.J. and Fahey, G.T. (1997). Utility of the white gene in estimating phylogenetic relationships among mosquitoes (*Diptera: Culicidae*). *Mol. Biol. Evol.* 14, 442-454.

Bogh, C., Clarke, S.E., Pinder, M., Sanyang, F. and Lindsay, S.W. (2001). Effect of passive Zooprophylaxis on malaria transmission in The Gambia. *J. Med. Entomol.* 38, 822-828.

Bogh, C., Clarke, S.E., Walraven, G.E.L. and Lindsay, S.W. (2002). Zooprophylaxis, artefact or reality? A paired-cohort study of the effect of passive zooprophylaxis on malaria in The Gambia. *Trans. Roy. Soc. Trop. Med. Hyg.* 96, 593-596.

Bouma, M. and Rowland, M. (1995). Failure of passive zooprophylaxis: cattle ownership in Pakistan is associated with a higher prevalence of malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 89, 351-353.

Bulterys, P.L., Mharakurwa, S. and Thuma, P.E. (2009). Cattle, other domestic animal ownership, and distance between dwelling structures are associated with reduced risk of recurrent *Plasmodium falciparum* infection in southern Zambia. *Trop. Med. Int. Health* 14, 522-528.

Chirebvu, E. and Chimbari, J.M. (2016). Characterization of an indoor-resting population of *Anopheles arabiensis* (Diptera: Culicidae) and the implications on malaria transmission in Tubu village in Okavango Sub-district, Botswana. *J. Med. Vet. Entomol.* 24, 569-576.

Coluzzi, M., Sabatini, A., Petrarca, V. and Di Deco M.A. (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. Roy. Soc. Trop. Med. Hyg.* 73, 483-497.

Deressa, W., Ali, A. and Berhane Y. (2007). Household and socioeconomic factors associated with childhood febrile illnesses and treatment seeking behavior in an area of epidemic malaria in rural Ethiopia. *Trans. Roy. Soc. Trop. Med. Hyg.* 101, 939-947.

Duchemin, B.J., Pock Tsy, J-M.L., Rabarison, P., Roux J., Coluzzi M. and Costantini C. (2001). Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odor-baited entry traps. *Med. Vet. Entomol.*15, 50-57.

Faye, O., Konate L., Mouchet J., Fontenille D., Sy N., Hebrard G. and Herve J.P. (1997). Indoor resting by outdoor biting females of *Anopheles gambiae* complex (*Diptera: Culicidae*) in the Sahel of northern Senegal. *J. Med. Vet. Entomol.* 34, 285-289.

Foley, D.H., Bryan J.H. and Lawrence G.W. (2000). The potential of ivermectin to control the malaria vector *Anopheles farauti*. *Trans. Roy. Soc. Trop. Med. Hyg.* 94, 625-628.

Fontenille, D., Lochouarn L., Diatta M., Sokhna C., Dia I., Diagne N., Lemasson J.J., Ba K., Tall A., Rogier C. and Trape J.F. (1997). Four years entomological study of the transmission of seasonal malaria in Senegal and the bionomics of *Anopheles gambiae* and *An. arabiensis*. *Trans. Roy. Soc. Trop. Med. Hyg.* 91, 647-652.

Fornadel, C.M., Norris L.C., Glass G.E. and Norri D.E. (2010). Analysis of *Anopheles arabiensis* blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. *Am. J. Trop. Med. Hyg. 83*, 848-853.

Francis, E.G. C. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites Vectors* 3,5.

Franco, O.M., Gomes, M.G., Rowland M., Coleman G.P. and Davies R.C. (2014). Controlling malaria using livestock-based interventions: a one health approach. *PLoS One* 9, e101699.

Fritz, M.L., Siegert, P.Y., Walker E.D., Bayoh H.M.N., Vulule J.R. and Miller J.R. (2009). Toxicity of blood meals from ivermectin-treated cattle to *Anopheles gambiae s.l. Ann. Trop. Med. Parasitol. 103*, 539-547.

Ghebreyesus, T.A., Haile M., Witten K.H., Getachew A.H., Yohannes M., Lindsay S.W. and Byass P. (2000). Household risk factors for malaria amongst children in the Ethiopian highlands. *Trans. Roy. Soc. Trop. Med. Hyg.* 94, 17-21.

Gillies, M.T. and Coetzee, M. (1987). A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). *S. Afr. Inst. Med. Res.* 55, 1-143.

Gimnig, J.E., Ombok, M., Kamau, L. and Hawley, W.A. (2001). Characteristics of larval Anopheline (*Diptera: Culicidae*) habitats in western Kenya. *J. Med. Entomol.* 38, 282-288.

Githeko, A.K., Adungo, N.I., Karanja, D.M., Hawley, W.A., Vulule, J.M., Seroney, I.K., Ofulla, A.V.O., Atieli, F.K., Ondijo, S.O., Genga, I.O., Odada, P.K., Situbi, P.A. and Oloo, J.A. (1996a). Some observations on the biting behavior of *Anopheles gambiae s.s*., *Anopheles arabiensis*, and *Anopheles funestus* and their implications for malaria control. *Exp Parasitol* 82, 306-315.

Githinji, S., Herbst, S. and Kistemann, T. (2009). The human ecology of malaria in a highland region of South-West Kenya. *Schattauer* 48, 451-453.

Govere, J., Braack, L.E., Durrheim, D.N., Hunt, R.H. and Coetzee, M. (2001). Repellent effects on *Anopheles arabiensis* biting humans in Kruger Park, South Africa. *Med. Vet. Entomol.* 15, 287-292.

Habtewold, T., Walker, A.R., Curtis C.F., Osir E.O. and Thapa N. (2001). The feeding behavior and *Plasmodium* infection of *Anopheles* mosquitoes in southern Ethiopia in relation to use of insecticide-treated livestock for malaria control. *Trans. Roy. Soc. Trop. Med. Hyg.* 95, 584-586.

Habtewold, T., Prior, A., Torr, S.J. and Gibson, G. (2004). Could insecticide-treated cattle reduce Afrotropical malaria transmission? Effects of deltamethrin-treated zebu on *Anopheles arabiensis* behavior and survival in Ethiopia. *Med. Vet. Entomol.*18, 408-417.

Hadis, M., Lulu, M., Makonnen, Y. and Asfaw, T. (1997). Host choice by indoor-resting *Anopheles arabiensis* in Ethiopia. *Trans. Roy. Soc. Trop. Med. Hyg.* 91, 376-378.

Harbach, R.E. (2004). The classification of genus Anopheles (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bull. Entomol. Res.* 94, 537-553.

Harbach, R.E. and Kitching, I.J. (1998). Phylogeny and classification of the Culicidae (Diptera). *Syst. Entomol.* 23, 327-370.

Hassanali, A., Nedorezov, L.V. and Sadykov A.M. (2008). Zooprophylactic diversion of mosquitoes from human to alternative hosts: A static simulation model. *Ecol. Model.* 212, 155-161.

Hewitt, S. and Rowland M. (1999). Control of zoophilic malaria vectors by applying pyrethroid insecticides to cattle. *Trop. Med. Int. Health* 4, 481-486.

Idrees, M. and Jan, A.H. (2001). Failure of zooprophylaxis: cattle ownership increase rather than reduce the prevalence of malaria in District Dir, N.W.F.P. of Pakistan. *J. Med. Sci.* 1, 52-54.

Iwashita, H., Dida, O.G., Sonye, O.G., Sunahara, T., Futami, K., Njenga, M.S., Chaves, F.L. and Minakawa, N. (2014). Push by a net, pull by a cow: can zooprophylaxis enhance the impact of insecticide treated bed nets on malaria control? *Parasites Vectors* 7, 52.

Kaburi, J.C., Githuto, J.N., Muthami, L., Ngure, P.K., Mueke, J.M. and Mwandawiro, C.S. (2009). Effects of long-lasting insecticidal nets and zooprophylaxis on mosquito feeding behavior and density in Mwea, central Kenya. *J. Vector Borne Dis.* 46, 184-190.

Karunamoorthi, K., Ilango, K. and Murugan, K. (2010). Laboratory evaluation of traditionally used plant-based insect repellent against the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae). *Parasitol. Res.* 106, 1217-1223.

Kawaguchi, I., Sasaki, A. and Mogi, M. (2004). Combining zooprophylaxis and insecticide spraying: a malaria-control strategy limiting the development of insecticide resistance in vector mosquitoes. *Proc. R. Soc. B. Biol. Sci.* 271, 301-309.

Kent, J.R., Thuma, E.P., Mharakurwa, S. and Norris, E.D. (2007). Seasonality, blood feeding behavior, and transmission of *plasmodium falciparum* by A*nopheles arabiensis* after an extended drought in southern Zambia. *Am. J. Trop. Med. Hyg.* 76, 267-274.

Kibret, S., Alemu, Y., Boelee, E., Tekie, H., Alemu, D. and Petros, B. (2010). The impact of a small-scale irrigation scheme on malaria transmission in Ziway area, Central Ethiopia. *Trop. Med. Int. Health* 15, 41-50.

Killeen, F.G. and Smith, A.T. (2007). Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. *Trans. Roy. Soc. Trop. Med. Hyg.* 101, 867-880.

Killeen, F.G., Ellis, M. F., Foy, D.B., Bøgh, C. and Beier, C.J. (2001). The availability of potential hosts as a determinant of feeding behaviors and malaria transmission by African mosquito populations. *Trans. Roy. Soc. Trop. Med. Hyg.* 95, 469-476.

Krzywinski, J. and Besansky, N.J. (2003). Molecular systematics of Anopheles: from subgenera to subpopulations. *Annu. Rev. Entomol.* 48, 111-139.

Levens W. (2013). *Mathematical modelling of co-application of long lasting insecticidal nets and insecticides zooprophylaxis against the resilience of Anopheles arabiensis for effective malaria prevention.* MSc. Thesis. University of Dar es Salaam. Dar es salaam.

Lyimo, I.N., Kija, R.N., Monica, W.M. Ally, A.D., Dickson, D.M., Nuru, S.N., Dickson, W. L. and Ladslaus L.M. (2012). Does cattle milieu provide a potential point to target wild exophilic *Anopheles arabiensis* (Diptera: Culicidae) with entomopathogenic fungus? A bioinsecticide zooprophylaxis strategy for vector control. *J. Parasitol. Res.* doi:10.1155/2012/280583.

Lyons, C.L., Coetzee, M., Terblanche, J.S. and Chown, S.L. (2012). Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malar. J.* 11, 226 doi: 10.1186/1475-2875-11-226

Mahande, A.M., Mosha, F.W., Mahande, J. M. and Kweka, E.J. (2007a). Feeding and resting behavior of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malar. J.* 6, 100 doi:10.1186/1475-2875-6-100.

Mahande, A.M., Mosha, F.W., Mahande, J.M. and Kweka, E.J. (2007a). Role of cattle treated with deltamethrine in areas with a high population of *Anopheles arabiensis* in Moshi, Northern Tanzania. *Malar. J.* 6, 109 doi:10.1186/1475-2875-6-109.

Massebo, F., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2015). Zoophagic behaviour of anopheline mosquitoes in southwestern Ethiopia: opportunity for malaria vector control. *Parasites Vectors* 8, 645 DOI: 10.1186/s13071-015-1264-9.

Mathys, T. (2010). Effectiveness of zooprophylaxis for malaria prevention and control in settings of complex and protracted emergency. *Resilience: interdiscipl. perspec. sci. humanitar.* 1, 1-26.

Mayagaya, S.V., Nkwengulila, G., Lyimo, N.I., Kihonda, J., Mtambala, H., Ngonyani, H., Russell, L.T. and Ferguson, M.H. (2015). The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania**.** *Malar. J.* 14, 17.

Mendis, C., Jacobsen, J.L., Gamage-Mendis, A., Bule, E., Dgedge, M., Thompson, R., Cuamba, N., Barreto, J., Begtrup, K., Sinden, R.E. and Hogh B. (2000). *Anopheles arabiensis* and *An. funestus* are equally important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. *Med. Vet. Entomol.*14, 171-180.

Mereta, T.S., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, O.S., Legesse, W., De Meester, L., Goethals, L.M.P. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites Vectors* 6, 320.

Minakawa, N., Githure, I.J., Beier, C.J. and Yan, G. (2001). Anopheline mosquito survival strategies during the dry period in Western Kenya. *J. Med. Entomol.* 38, 388-392.

Mnzava, A.E., Rwegoshora, R.T., Wilkes, T.J., Tanner, M. and Curtis, C.F. (1995). *Anopheles arabiensis* and *An. gambiae* chromosomal inversion polymorphism, feeding and resting behavior in relation to insecticide house spraying in Tanzania. *J. Med. Vet. Entomol.* 9, 316-324.

Muriu, M.S., Muturi, J.E., Shililu, I.J., Mbogo, M.C., Mwangangi, M.J., Jacob, G.B., Irungu, W.L., Mukabana, W.R., Githure, I.J. and Novak, J.R. (2008). Host choice and multiple bloods feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malar. J.* 7, 43.

Musa, J., Pinder, M., Drakeley, C.J., Nwakanma, D.C., Jallow, E., Bogh, C., Lindsay, S.W. and Conway, D.J. (2008). Dry season ecology of *Anopheles gambiae* complex mosquitoes in the Gambia*. Malar. J.* 7, 156.

Mutero, C.M., Ng'ang'a, P.N., Wekoyela, P., Githure, J. and Konradsen, F. (2004). Ammonium sulphate fertilizer increases larval populations of *Anopheles arabiensis* and culicine mosquitoes in rice fields. *Acta Trop.* 89, 187-192.

Mwangangi, M.J., Muturi, E.J., Shililu, J., Muriu, S.M., Jacob, B., Kabiru, E.W., Mbogo, C.M., Githure, J. and Novak R. (2006). Survival of immature *Anopheles arabiensis* (Diptera: Culicidae) in aquatic habitats in Mwea rice irrigation scheme, central Kenya. *Malar. J.* 5, 114.

Nah, K., Kima, Y. and Lee, J.M. (2010). The dilution effect of the domestic animal population on the transmission op *P. vivax* malaria. *J Theor. Biol.* 226, 299-306.

Padonou, G.G., Gbedjissi, G., Yadouleton, A., Azondekon, R., Razack, O., Oussou, O., Gnanguenon, V., Rock, A., Sezonlin, M. and Akogbeto, M. (2012). Decreased proportions of indoor feeding and endophily in *Anopheles gambiae s.l.* populations following the indoor residual spraying and insecticide-treated net interventions in Benin (West Africa). *Parasite Vector* 5, 262-272.

Palsson, K., Thomas, G.T., Dias, J.F., Laugen, A.T. and Bjorkman A. (2004). Endophilic Anopheles mosquitoes in Guinea Bissau, West Africa, in relation to human housing conditions. *J. Med. Entomol.* 41, 746-752.

Pates, H.V., Takken, W., Stuke, K. and Curtis, C.F. (2001). Differential behavior of *Anopheles gambiae* sensu strictu (*Diptera: Culicidae*) to human and cow odors in the laboratory. *Bull. Entomol. Res.* 91, 289-296.

Pates, H. and Curtis, C. (2005). Mosquito behavior and vector control. *Annu. Rev. Entomol.* 50,53-70.

Reddy, M.R., Overgaard, H.J., Abaga, S., Reddy, V.P., Caccone, A., Kiszewski, A.E. and Slotman, M.A. (2011). Outdoor host seeking behavior of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar. J.* 10, 184 doi: 10.1186/1475-2875-10-184.

Rich, S.M. and Xu G. (2011). Resolving the phylogeny of malaria parasites. *Proc. Natl. Acad. Sci.* 108, 12973-12974.

Robert, V., and Carnevale, P. (1991). Influence of deltamethrin treatment of bed nets on malaria transmission in the Kou valley, Burkina Faso. *Bull. World Health Organ.* 69, 735-740.

Rowland, M., Durrani, N., Kenward, M., Mohammed, N., Urahman, H., and Hewitt, S. (2001). Control of malaria in Pakistan by applying deltamethrin insecticide to cattle: a community-randomized trial. *Lancet* 357, 1837-1841.

Rúa, G.L., Quinones, M.L., Velez, I.D., Zuluaga, J.S., Rojas, W., Poveda, G. and Ruiz, D. (2005). Laboratory estimation of the effects of increasing temperatures on the duration of

gonotrophic cycle of *Anopheles albimanus* (*Diptera: Culicidae*). *Memorias do Instituto Oswaldo Cruz.* 100, 515-520.

Russell, T.L., Govella, N.J., Azizi, S., Drakeley, C.J., Kachur, S.P. and Killeen, G.F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar. J.* 10, 80 doi: 10.1186/1475-2875-10-80.

Sallum, A.M., Schultz, T.R., Foster, P.G., Aronstein, K., Wirtz, R.A. and Wilkerson, R.C. (2002). Phylogeny of *Anophelinae* (*Diptera: Culicidae*) based on nuclear ribosomal and mitochondrial DNA sequences. *Sys. Entomol.* 27, 361-382.

Saul, A. (2003). Zooprophylaxis or zoopotentiation: the outcome of introducing animals on vector transmission is highly dependent on the mosquito mortality while searching. *Malar. J.* 2, 32 doi: 10.1186/1475-2875-2-32.

Seyoum, A., Balcha, F., Balkew, M., Ali, A. and Gebre-Michael, G. (2002). Impact of cattle keeping on human biting rate of *Anopheline* mosquitoes and malaria transmission around Ziway, Ethiopia. *East. Afr. Med. J.* 79, 485-490.

Sinka, M.E., Bangs, M.J., Mangiun, S., Rubio-Palis, Y., Chareoviriyaphap, T., Coetzee, M., Mbogo, C.M., Haminway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Burkot, T.R., Harbach, R.E. and Hay, S.I. (2012). A global map of dominant malaria vectors. *Parasites Vectors* 5, 69 doi: 10.1186/1756-3305-5-69.

Taye, A., Hadisa, M., Adugnaa, N., Tilahuna, D. and Wirtzb, R.A. (2006). Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis* from Sille, Ethiopia. *Acta Trop.* 97, 50-54.

Temu, E.A., Coleman, M., Abilio, A.P. and Kleinschmidt, I. (2012). High prevalence of malaria in Zambezia, Mozambique: the protective effect of IRS versus increased risks due to pig-keeping and house construction. *PLoS One* 7: e31409.

Theobald, V.F., (1901). *A monograph of the culicidae, or mosquitoes* 1. London: British Museum (Natural History). 1901: ISBN 978-1178519037.

Tirados, I., Costantini, C., Gibson, G. and Torr S.J. (2006). Blood-feeding behavior of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med. Vet. Entomol.* 20, 425-437.

Tirados, I., Gibson, G., Young, S. and Torr, S.J. (2011). Are herders protected by their herds? An experimental analysis of zooprophylaxis against the malaria vector *Anopheles arabiensis*. *Malar. J.* 10, 68 doi:10.1186/1475-2875-10-68.

Tuna, N., Githeko, A.K., Nakayama, T., Minakawa, N., Takagi, M. and Yan, G.Y. (2006). The association between the phytoplankton, *Rhopalosolen* species (*Chlorophyta; Chlorophyceae*), and *Anopheles gambiae* sensu lato (*Diptera: Culicidae*) larval abundance in western Kenya. *Ecol Res.* 21, 476-482.

WHO. (1982). World health organization. Manual on environmental management for mosquito control with special emphasis on malaria vectors. World health organization, Geneva. 1982.

WHO. (2015). World malaria report. World health organization, Geneva. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed: 04/04/2016].

Yamamoto S.S., Louis V.R., Sie A. and Sauerborn R. (2009). The effects of zooprophylaxis and other mosquito control measures against malaria in Nouna, Burkina Faso. *Malar. J.* 8, 283 doi:10.1186/1475-2875-8-283.

Ye-Ebiyo, Y., Pollack, R. and Spielman, A. (2000). Enhanced development in nature of larval *Anopheles arabiensis* mosquitoes feeding on maize pollen. *Am. J. Trop. Med. Hyg.* 63, 90-93.

Ye-Ebiyo, Y., Pollack, R.J., Kiszewski, A. and Spielman, A. (2003). Enhancement of development of larval *Anopheles arabiensis* by proximity to flowering maize in turbid water and when crowded. *Am. J. Trop. Med. Hyg.* 68, 748-752.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Leen, D., Tessema, D.A., Getachew, Y., Marc, C., Duchateau, L. and Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PLoS One* 6, e16066. doi:10.1371/journal.pone.0016066.

Yohannes, M. and Boelee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Med. Vet. Entomol.* 26, 103-105.

Chapter 4

Evaluation of the efficacy of DDT indoor residual spraying and long-lasting insecticidal nets against insecticide resistant populations of *Anopheles arabiensis* Patton (Diptera: Culicidae) from Ethiopia using experimental huts

Adapted from

Asale, A., Getachew, Y., Hailesilassie, W., Speybroeck, N., Duchateau, L. and Yewhalaw, D. (2014). Evaluation of the efficacy of DDT indoor residual spraying and long-lasting insecticidal nets against insecticide resistant populations of *Anopheles arabiensis* Patton (Diptera: Culicidae) from Ethiopia using experimental huts**.** *Parasites Vectors* 7:131.

4.1 Abstract

Background: Indoor Residual Spraying (IRS) and Long-Lasting Insecticidal Nets (LLIN) are major malaria vector control tools in Ethiopia. However, recent reports from the different parts of the country showed that the population of *Anopheles arabiensis*, the principal malaria vector, developed resistance to most families of insecticides recommended for public health use which may compromise the efficacy of both these key vector control interventions. Thus, this study evaluated the efficacy of IRS and LLINs against resistant population of *Anopheles arabiensis* using experimental huts.

Methods: The susceptibility status of the *Anopheles arabiensis* population for DDT, deltamethrin, malathion, lambda-cyhalothrin, fenitrothion and bendiocarb was assessed using WHO test kits. The efficacy of LLIN (PermaNet ® 2.0) was evaluated using the WHO cone bioassay. Moreover, the effect of the observed resistance against the existing malaria vector control interventions (IRS and LLINs) was assessed using experimental huts from August to November 2011.

Results: The findings of this study revealed that the *Anopheles arabiensis* population was resistant to DDT (1.3%), deltamethrin (18.8%), malathion (72.5%) and lambda-cyhalothrin (36.3%) but susceptible to fenitrothion and bendiocarb with mortality rates of 98.81% and 97.5%, respectively. The bio-efficacy test of LLIN (PermaNet ® 2.0) against *Anopheles arabiensis* revealed that the mosquito population showed moderate knockdown (64%) and mortality (78%). Moreover, mosquito mortalities in sprayed huts and in huts with LLINs were not significantly different ($p > 0.05$) from their respective controls.

Conclusion: The evaluation of the efficacy of IRS and LLINs using experimental huts showed that both vector control tools had only low to moderate efficacy against the *Anopheles arabiensis* population from Ethiopia. Thus, there is a need for new alternative vector control tools and for the implementation of appropriate insecticide resistance management strategies as part of integrated vector management by the national malaria control program.

Keywords: Ethiopia, *Anopheles arabiensis*, insecticide resistance, experimental huts, and Long-Lasting Insecticidal Nets, Indoor Residual Spraying

4.2 Introduction

Malaria is endemic in 90 countries in tropical and subtropical zones (WHO, 2012). It remains one of the greatest health threats in Sub-Saharan Africa with high mortality and morbidity especially in children under the age of five years. There were globally about 219 million cases and an estimated 660,000 deaths due to malaria with about 90% of these cases occurring in Africa (WHO, 2012; Vannice et al., 2012).

In Ethiopia, malaria is seasonal in most parts of the country, with unstable transmission resulting in malaria epidemics. Malaria incidence decreased between 2004 and 2008, but in recent years malaria admissions increased, with the highest rate observed in 2011. In the aforementioned year only, 1,480,360 cases were observed of which 814,547 (55%) were due to *Plasmodium falciparum* and 665, 813 (45%) due to *Plasmodium vivax*. The disease prevalence varies across regional states ranging from 0.5% to 2.5% (WHO, 2012; FMoH, 2012). *Anopheles arabiensis,* a member of the *An. gambiae* complex, is the major vector in the country. Other anophelines which occur in Ethiopia are *An. funestus* group, *An. pharoensis* and *An. nili*. *An. funestus* and *An. pharoensis* are considered to be secondary vectors (Gebremariam, 1988; Tulu, 1993). Long-lasting Insecticidal Nets (LLINs), Indoor Residual Spraying (IRS) and environmental management are the most widely used tools for malaria vector control (WHO, 2010; WHO, 2007; WHO, 2006). Despite reports demonstrating the efficacy of both ITNs and IRS for curbing malaria incidence (Guyatt et al., 2002) insecticide resistance in malaria vectors threatens the success of malaria vector control programs in Sub-Saharan Africa (N'Guessan et al., 2007). If current trends continue, insecticide resistance may compromise control as it did in the last era of malaria eradication in the 1950's and 60's (Kelly-Hope et al., 2008). Given the limited number of available insecticides, i.e., only 12 insecticides belonging to 4 classes of insecticides (pyrethroids, organophosphates, carbamates and organochlorine) for IRS, and only one insecticide class (Pyrethroids) for ITNs (Najera and Zaim, 2001) the resistance related to these insecticides has become a limiting factor for malaria vector control. Following reports of DDT resistance undermining malaria vector control efforts (Hemingway and Ranson, 2000), the controversy around the use of DDT shifted the attention to the use of Pyrethroids which are considered to be less toxic to humans and other non-target organisms (Bouwman, 2000). Despite, Pyrethroids display better exito-repellent properties and faster killing effects than other insecticide classes, resistance to pyrethroids has emerged spreading rapidly and constituting a serious threat to malaria control initiatives (Etang et al., 2004).

In Ethiopia, LLINs and IRS are the two key vector control interventions. However, insecticide resistance, which became widespread in malaria vectors in western, southern, central and eastern Africa in recent years (Koffi et al., 2013; Ahoua-Alou et al., 2012; WHO, 2010; Yewhalaw et al., 2010), is a major challenge in malaria vector control. *An. arabiensis* has developed resistance against most insecticide families (organochlorines, organophosphates and pyrethroids) commonly used in public health (Balkew et al., 2010; Massebo et al., 2013; Abate and Hadis, 2011). The west African *kdr* (L1014F) mutation was also reported in population of *An. arabiensis* from the different parts of the country with an allelic frequency of 95-100 % (Balkew et al., 2012; Yewhalaw et al., 2011; Fettene et al., 2013). Moreover, pre-exposure of *An. arabiensis* from southwestern Ethiopia to piperonylbutoxide (PBO) significantly increased the susceptibility of the population to both permethrin and deltamethrin, indicating the possible involvement of metabolic resistance in addition to the previously described *kdr* resistance (Yewhalaw et al., 2012).

Despite the high coverage of IRS and scaling up of LLINs, there is no documented information yet on the effect of insecticide resistance on the existing malaria vector control interventions in Ethiopia. Thus, the aim of this study was to assess the impact of insecticide resistance on malaria vector control interventions (LLINs and IRS) in Ethiopia.

4.3 Materials and methods

4.3.1 Study area and period

This study was conducted from August to November 2011 around the Gilgel-Gibe hydropower dam area, southwestern Ethiopia. The Gilgel-Gibe hydroelectric power dam is one of the largest hydropower dams in Ethiopia. It produces about 184MW and is located 260km south west of Addis Ababa, in Oromia regional state, southwestern Ethiopia. It has become operational in 2004. The region is located between latitudes $7^0$42’50” N and $07^0$53’50” N and longitudes $37^0$11’22” E and $37^0$20’36” E, at an altitude ranging from 1,672-1,864m above sea level. The region has a sub-humid, warm to hot climate, receives between 1,300 and 1,800 mm of rain annually and has a mean annual temperature of 19^{o}C. The rainfall is divided in to the long rainy season starting in June and extending up to September, and the short rainy season beginning in March and extending to April/May.

4.3.2 Insecticide bioassays

Anopheline mosquito larvae were collected by dipping from a range of breeding sites (road paddies, brick pits, pools, marshes, streams, surface water harvest, ditches, dam reservoir shore, and pits dug for plastering traditional tukuls) around Osso Bille village, Asendabo, where the experimental huts were established. Mosquito larvae were reared to adults in the field Vector Biology Laboratory, Jimma University under standard conditions (temperature 25 ± 2 ^{o}C, relative humidity 80 ± 4%). The larvae were fed with dog biscuits and brewery yeast (Gerberg et al., 1994). Two to three days old, non-blood fed female mosquitoes were exposed to insecticide impregnated papers using the insecticides DDT (4%), deltamethrin (0.05%), malathion (5%), lambdacyalothrin (0.05%), fenitrothion (1.0%) and bendiocarb (0.1%) following the WHO standard assay (WHO, 2006; WHO, 1998). The insecticide impregnated and control papers were obtained from the WHO collaboration Centre, Vector Control Research Unit, School of Biological Sciences, Penang, Malaysia. Batches of 20–25 mosquitoes (four replicates) were exposed in test kit tubes for all bioassays for one hour against the four classes of insecticides and knockdown was recorded at 10, 15, 20, 30, 40, 50, and 60 minutes. An equal number of mosquitoes (one replicate) was exposed to the corresponding control papers impregnated with resila oil (organochlorine control), olive oil (organophosphate/carbamate control), and silicone oil (pyrethroid control). After one hour, mosquitoes were transferred into holding tubes and provided with 10% sucrose solution with cotton pads. Mortality was recorded 24 hours post exposure.

4.3.3 LLIN sample preparation and WHO cone assays

Three rectangular nets of PermaNet® 2.0 and three untreated nets to be used as a negative control was purchased from the local market in Ethiopia. The production date and batch number of all nets were recorded. Three sub-samples per net (one from the roof and two from each long side of the net) were taken from each net and prepared for standard LLINs cone tests by cutting 30 cm x 30 cm pieces. Each sub-sample was rolled up in aluminum foil, labeled (by net type, net number and sample area) and kept individually in a refrigerator prior to the assay. For each individual sub-sample, four cone tests were conducted sequentially following the standard WHO procedure (WHO, 2006). Five non blood-fed, two to three days old, female mosquitoes were introduced into each cone and exposed to each bed net sample for 3 minutes before being transferred to paper cups and held with access to 10% sugar solution. Knockdown (KD) was recorded at 1, 3, 5, 10, 15, 30, 45 and 60 minutes and mortality (MT) was recorded 24 hours

post-exposure. A total of 180 mosquitoes were tested (20 mosquitoes x 3 subsamples x 3 nets). Replicates of cone assays with sub-samples taken from untreated nets were also conducted concurrently as a negative control. Mortality was corrected using Abbott's formula when mortality in the control exceeded 5% (Abbott, 1925). Bioassays were carried out at a temperature of 27±2°C and relative humidity of 80±4%.

4.3.4 Establishment of experimental huts

Four experimental huts, each with one room and a large screened veranda trap were established approximately 500m West of the Gilgel-Gibe reservoir shore, southwestern Ethiopia, and used for the evaluation of the efficacy of IRS and LLINs (Figure 4.1). The experimental huts were constructed following the WHO guidelines (WHO, 2006). The dimensions of the hut were 2.5m wide, 2.5m long and 3m high while that of the verandah trap was 2m long, 1.5m wide and 1.5m high being projected from the back wall of each hut. The walls of the huts were constructed from plywood and wooden frame for easy manipulation and transportation. The huts were covered with red brown colored polyethylene plastic on the outside in order to simulate the wall color of local tukuls. The roof was made of corrugated iron sheet. The slits were constructed from pieces of plywood, fixed at an angle of 45^0 to create a funnel of 1 cm between slits. The window slits were designed in such a way that the mosquitoes could not escape once they entered the hut. The window slits were made in such a way to allow those mosquitoes fly upward to enter into the huts through the open space and those which fly downward to exit; Consequently, the design of the slits precluded influx of mosquitoes into and out of the experimental huts. Each hut had a veranda trap made of iron meshes (22 mm) for trapping exophilic mosquitoes. Mosquitoes inside the hut could only exit via the veranda which was shut down by lowering a curtain separating the sleeping room from the veranda. Each hut had a ceiling made of white sheets. A gutter was dug around each hut and filled with water to exclude ants and other scavenger arthropods which otherwise could carry off dead mosquitoes from the huts during the night. Each night white sheets were spread on the floor of the experimental hut to collect knocked down and/ or dead mosquitoes.

Figure 4.1 Field experimental huts

4.3.5 Treatment arms and sleepers' rotation

The treatments for this trial were DDT for IRS and PermaNet 2.0 for LLINs. DDT was obtained from Adam Tulu Pesticide Processing S.C. Addis Ababa, Ethiopia and a WHOPES approved LLIN (PermaNet ® 2.0) made of multifilament polyester fibers, factory-coated with a wash resistant formulation of deltamethrin at a target dose of 55mg/m^2 was obtained from local market. A dose of 2g/m^2 DDT wetable powder (WP) was sprayed onto interior walls of one of the four huts, randomly chosen, using a Hudson compression sprayer equipped with a flat fan nozzle (WHO, 2006). The untreated bed net is made of white 100-denier polyester multifilament net (Siamdutch Mosquito Netting Co., Ltd, Bangkok, Thailand). Six holes of 4 cm × 4 cm were made in each mosquito net, two in each long side and one at each shorter side to simulate the conditions of a torn net and to ensure that the insecticide, rather than the net, effectively prevents mosquito bites. Huts assigned for IRS treatment were fixed throughout the study according to the WHO guideline as the IRS treatment could not be rotated due to residual effect of DDT (WHO, 2006). The LLIN, untreated net and unsprayed hut treatments, however, were rotated weekly between huts, in a 3x3 Latin Square Design (LSD), with week and hut being the rows and the columns of the Latin square.

A baseline study was conducted in July, 2011 to evaluate the attractiveness of the experimental huts. The trial lasted for four weeks from July 20, 2011 to August 24, 2011. Eight teams of two people served as volunteer sleepers and each team was rotated between treatments on successive nights within a week to avoid possible bias which could arise due to individual attractiveness to mosquitoes. The teams slept in the huts from 19:00 h to 06:00 h each night. Informed written consent was obtained from each sleeper.

4.3.6 Mosquito collection, identification and determination of IRS and LLIN efficacy

Anopheline mosquitoes were collected each morning from 06:00 h to 7:00 h from inside bed nets, floors, walls, ceilings and veranda traps of each experimental hut using mouth aspirators and torches. Then the collected mosquitoes were recorded as dead or alive. Live mosquitoes were held in paper cups and supplied with 10% sucrose solution. The collected mosquitoes were transported to Asendabo Vector Biology Laboratory, Jimma University, where mosquitoes were sorted by genus, sex and morphologically identified using taxonomic keys (Gillies and Coetzee, 1987). Mosquitoes were also scored for their physiological state as unfed, fed, half gravid and gravid. Delayed mortality was recorded after 24 h.

To evaluate the efficacy of ITNs and IRS against the resistant population of *An. arabiensis*, different entomological parameters (deterrence, exit, blood feeding inhibition and mortality rates) were derived from basic measurements following an established formula (WHO, 2006). The basic measurements considered were: number of collected female mosquitoes, blood-fed female mosquitoes and dead female mosquitoes, denoted respectively by N, B, and D. These basic measurements were indexed to denote the collection place (first sub-index) and the treatment (second sub-index). For location, ‘h’ refers to collection from inside the hut, whereas ‘e’ refers to the verandah trap, and finally ‘t’ is the sum of the two (‘h’+’e’).

For treatment, ‘c’ refers to unsprayed hut, ‘i’ to sprayed hut (IRS), ‘u’ to untreated bed net and ‘b’ to treated bed net (LLIN).

In comparing IRS with its control, the deterrence rate for IRS is given by

$$\text{Deterrence rate IRS} = 100 \times \frac{(Nt,c - Nt,I)}{Nt,c}$$

with

$N_{t,c}$ = the sum total no of mosquitoes collected from a hut and exit trap of unsprayed hut

$N_{t,I}$ = the sum total no of mosquitoes collected from a hut and exit trap of sprayed hut

whereas the deterrence rate for treated LLIN compared to its control is given by

$$\text{Deterrence rate LLIN} = 100 \times \frac{(Nt,c - Nt,I)}{Nt,c}$$

with

$N_{t,c}$ = the sum total no of mosquitoes collected from a hut and exit trap with untreated net

$N_{t,I}$ = the sum total no of mosquitoes collected from a hut and exit trap with LLIN

For a particular hut with treatment *j*, the entomological parameters are defined as

$$\text{Exit rate} = \frac{N_{e,j}}{N_{t,j}} \times 100$$

$$\text{Blood feeding inhibition rate} = \frac{B_{t,j}}{N_{t,j}} \times 100$$

$$\text{Mortality rate} = \frac{D_{t,j}}{N_{t,j}} \times 100$$

$$\text{Personal Protection (\%)} = 100 \times \frac{Bc - Bt}{Bc}$$

with

B_c= total no of blood-fed mosquitoes in the hut with untreated net

B_t= total no of blood-fed mosquitoes in hut with LLIN

and

$$\text{Killing effect (\%)} = 100 \times \frac{\text{(Dt-Dc)}}{\text{Ec}}$$

with

D_t = total no of mosquitoes dead in a hut with LLIN

D_c = total no of mosquitoes dead in a hut with untreated net

E_c = total no of mosquitoes entering a hut with untreated net

4.3.7 Data analysis

The LLIN and untreated bed net on the one hand, and sprayed and unsprayed hut on the other hand, were compared with one another with respect to blood feeding inhibition, exit and mortality rates. A linear fixed effects model was used including treatment and week as fixed effects. F-tests were performed at a global significance level of 5% but testing each of the two comparisons at the Bonferroni adjusted comparisons wise significance level of 2.5%. All analyses were done using SAS software package version 9.3 (SAS Institute Inc., Cary, NC, USA).

4.3.8 Ethical consideration

The study protocol was reviewed and approved by the research and ethics committee of Jimma University, Ethiopia.

4.4 Results

4.4.1 Insecticide and cone bioassays

The susceptibility status of population of *An. arabiensis* to five insecticides commonly used in malaria vector control in Ethiopia is shown in Table 4.1. Population of *An. arabiensis* showed reduced mortality to DDT, deltamethrin, lambda-Cyhalothrin and malathion; however, mosquito population was fully susceptible to fenitrothion and bendiocarb.

Exposure of mosquitoes to net sections of PermaNet ® 2.0 in cone bioassay test led to an observed average mortality of 64% and knock down of 78%, which is well below the required levels of 80% and 95%, respectively (Figure 4.2).

Table 4.1 Mean mortality rate of *Anopheles arabiensis* for six insecticides, southwestern Ethiopia

Type of insecticide	Exposed			Non-exposed		
	No. tested	No. dead	Mortality (%)	No. tested	No. dead	Mortality (%)
DDT (4%)	80	1	1.25	40	0	0.00
Deltamethrin (0.05%)	80	15	18.75	40	0	0.00
Malathion (5%)	80	58	72.50	40	2	5.00
Lambdacylothrin (0.05%)	80	29	36.25	40	0	0.00
Fenitrothion (1.0%)	84	83	98.81	40	0	0.00
Bendiocarb (0.1%)	80	78	97.50	40	0	0.00

4.4.2 Mosquito deterrence rate, personal protection and insecticidal effect

Overall, 2391 and 1023 anopheline and culicine mosquitoes were collected, respectively during the trial. Of the 2391 anopheline mosquitoes, 2209 (92.4%) belonged to *An. gambiae* s.l (presumably *An. arabiensis*) (Yewhalaw et al., 2010; Fettene et al., 2013), 160 (6.7%) to *An. coustani* and 22 (0.9%) to *An. pharoensis*. Of the total 2209 *An. arabiensis* collected, 479 (22%) were from DDT sprayed hut, 793 (36%) from unsprayed hut, 426 (19%) from huts with LLIN and the remaining 511 (23%) from hut with untreated net. The deterrence rate of DDT sprayed hut and a hut with LLIN was 39.6% and 16.6%, respectively. Moreover, personal protection in a hut with LLIN was over 21% against *An. arabiensis* as compared to a hut with untreated nets while the insecticidal effect in a hut with LLIN was 19.6%.

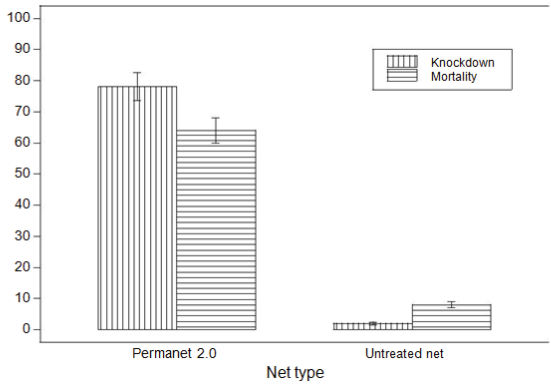

Figure 4.2 Mean percent knockdown and mortality of WHO cone bioassay test for permaNet 2.0 and untreated net, July-August, 2011, Jimma, southwestern Ethiopia

4.4.3 Mosquito mortality, blood feeding inhibition and exit rates

Mosquito blood feeding rates, exit rates and mortality rates of the 4 treatments are presented in Table 4.2. There was no significant difference ($p > 0.05$) in mosquito blood feeding rates between sprayed (76.1%) and unsprayed hut (80.3%) and between a hut with treated net (55.1%) and the hut with untreated net (58.9%). Moreover, the mean exit rate was similar ($P > 0.05$) for sprayed hut (48.6%) and unsprayed hut (42.3%) and between a hut with treated net (49.4%) and a hut with untreated net (41.4%). There was no significant ($P > 0.05$) difference in mosquito mortality between sprayed and unsprayed hut nor between a hut with LLIN and a hut with untreated net.

Table 4.2 Mean blood feeding, exit rate and morta1ity rate of *Anopheles arabiensis*

Treatment	Blood feeding rate n (Mean ± SE)	Exit rate n (Mean ± SE)	Mortality rate n (Mean ± SE)
Sprayed hut	364 (76.1 ± 5.1)	233 (48.6 ± 3.9)	247 (51.5 ± 5.6)
Unsprayed hut	641 (80.8 ± 6.6)	335 (42.3 ± 4.8)	324 (40.8 ± 5.5)
Hut with LLIN	235 (55.14 ± 3.9)	210 (49.4 ± 4.8)	247 (58.0 ± 7.0)
Hut with untreated net	301 (58.90 ± 5.7)	211 (41.4 ± 5.2)	294 (57.50 ± 6.7)

4.5 Discussion

Insecticide resistance is a major impediment in malaria vector control. In this study we initially assessed the susceptibility status of field population of *An. arabiensis* using WHO susceptibility test kits and bio-efficacy of LLINS. We further assessed the impact of resistance on the existing vector control interventions (IRS and LLINs) using an experimental hut trial following the WHOPES guideline (WHO, 2006). The results of the WHO insecticide susceptibility test showed that population of *An. arabiensis* have developed resistance to DDT, deltamethrin, malathion, and lambda-Cyhalothrin but were still susceptible to fenitrothion and bendiocarb. Previous reports from Ethiopia also showed that *An. arabiensis* population has developed resistance against three classes of insecticides. Yewhalaw et al. (2010; 2011; 2012) reported that the population of *An. arabiensis* from southwestern Ethiopia had developed resistance to DDT, Permethrin, deltamethrin, and malathion but were still fully susceptible to propoxur. A similar study by Balkew et al. (2010) in villages of central, northern and south western Ethiopia showed that populations of *An. arabiensis* developed resistance to DDT, deltamethrin, lambda-cyhalothrin, malathion and Bendiocarb. Recently, Fetene et al. (2013) reported that population of *An. arabiensis* from the southern and northern parts of the country were resistant to DDT and malathion. Another study conducted by Massebo et al. (2013) around southern Ethiopia revealed that the population of *An. arabiensis* was resistant to lambda-cyhalothrin, cyfluthrin and alpha-cypermethrin, deltamethrin, and DDT. Another study conducted by Abate and Hadis (2011) in northern, northwestern, central and southern Ethiopia confirmed the development of high level pyrethroid and DDT resistance in population of *An. gambiae s.l.* Likewise a widespread pyrethroid resistance of *An. arabiensis* was reported from western Kenya (Kawada et al., 2011). In the same way a study carried out in two villages of Côte d'Ivoire confirmed that resistance had developed at various degrees in both regions (Koudou et al., 2010). Likewise, insecticide susceptibility test reports from Burkina Faso, Chad and Sudan showed that all mosquito populations of *An. gambiae s.l* from Burkina Faso, Chad and two of the four populations of *An. arabiensis* from Sudan were resistant to permethrin, deltamethrin, and DDT whereas the same population remained largely susceptible to fenitrothion and bendiocarb (Ranson et al., 2009).

The mortality and knockdown results from the WHO cone bioassay test revealed that unwashed PermaNet® 2.0 had a reduced efficacy, although it caused much higher mortality and knockdown rates compared to the untreated net. Previous studies from the same region showed that the *An. arabiensis* population has developed pyrethroid resistance (Yewhalaw et al., 2012).

The involvement of metabolic resistance in the population of *An. arabiensis* had been reported using synergists (Yewhalaw et al., 2012). Norris and Norris (2011) reported that the *An. arabiensis* population in Zambia showed resistance to DDT and 12% of the mosquitoes tested survived after exposure to ITNs. In agreement with this finding, the population of *An. arabiensis* from Tanzania (Okumu et al., 2012) showed resistance to PermaNet ®2.0 with mortality reduced from 92.8% in the first month to 83.3% after six months. Similar results were reported from a study carried out in Côte d'Ivoire (Koudou et al., 2011) with wild resistant *An. gambiae* mosquitoes showing a mean knockdown rate below 95% and a mean mortality rate below 80% for all treatment arms, with the exception of unwashed PermaNet® 3.0 which caused 95.8% knock down and 97.0% mortality.

There was a 39.6% reduction in deterrence rate of *An. arabiensis* in DDT sprayed huts when compared to unsprayed huts and a reduction of 16.6% of mosquito deterrence rate in huts with LLIN compared to huts with untreated net. In the same way a study conducted in Tanzania using experimental hut trials revealed that PermaNet® 2.0 resulted in a 21% reduction in deterrence rate of *An. arabiensis* population (Tungu et al., 2010). Likewise, another study from Burkina Faso using experimental huts documented that the entry rate of *An. gambiae s.s.* into huts with LLIN and insecticide treated plastic sheeting (ITPS) was reduced compared to untreated huts (Chandre et al., 2010). Another study conducted in Vietnam using experimental huts also revealed a 30.7% reduction in density of population of *An. epiroticus* entering in to hut treated with PermaNet® 2.0 (Van Bortel et al., 2009).

The mosquito feeding and exit rates were very similar in the sprayed and unsprayed huts, and also in the huts with LLIN and with an untreated net. This is consistent with the findings of Ngufor et al. (2011) from Benin who showed that induced exophily rates in *An. gambiae s.s*. between the huts with LLIN (PermaNet®2.0) and CTN compared to their untreated controls were similar. Corbel et al. (2010) also noted the absence of significant reduction in entry rate between LLIN and untreated nets in their experimental hut study in the village of Malanville, Benin. In our study, mosquito mortality rates between the sprayed hut and its control and between a hut with PermaNet® 2.0 and a hut with untreated net were similar. A similar study conducted in Côte d'Ivoire showed that both unwashed PermaNet® 2.0 and PermaNet® 3.0 caused significantly higher mosquito mortality as compared to their respective control (Koudou et al., 2011). A study from Vietnam indicated significantly higher mosquito mortality among the treatment arms (huts treated with PermaNet® 2.0, PermaNet® 3.0 and CTN) as compared to their control (Van Bortel et al., 2009).

In conclusion, the population of *An. arabiensis* around the Gilgel-Gibe dam, southwestern Ethiopia has developed resistance to organo-chlorines, organophosphates, and pyrethroids. The evaluation of IRS using DDT and LLINs (PermaNet ® 2.0) based on a trial with experimental huts further suggests that neither DDT nor LLIN can stand alone as vector control tool in the presence of the resistant mosquito population in the study region. Therefore, alternative new vector control tools should be put in place and an insecticide resistance management strategy plan should be developed and implemented. One possible option could be combining LLIN with IRS using a new insecticide of choice (e.g., bendiocarb) which could reduce vector-human contact in the study area. Furthermore, large scale field trial studies should be carried out in order to confirm whether the current vector control interventions, IRS and LLINs, are still effective in different regions of Ethiopia in the presence of resistant populations of *An. arabiensis*.

Acknowledgements

Jimma University VLIR-IUC program is highly acknowledged for logistic and financial support. The authors are grateful to Asendebo Vector Biology Laboratory staffs for their excellent technical support.

4.6 References

Abate, A. and Hadis, M. (2011). Susceptibility of *Anopheles gambiae s.l.* to DDT, malathion, permethrin and deltamethrin in Ethiopia. *Trop. Med. Int. Health* 16, 486-491.

Abbott, W.S. (1925). A method for computing the effectiveness of insecticides. *J. Econ. Entomol.* 18, 265-267.

Ahoua-Alou, P.L., Koffi, A.A., Adja, A.M., Assi, B.S., Kouassi, K.P. and N'Guessa, R. (2012). Status of pyrethroid resistance in *Anopheles gambiae s. s.* M form prior to the scaling up of long-lasting insecticidal nets (LLINs) in Adzopé, Eastern Côte d'Ivoire. *Parasites Vectors* 5, 289.

Balkew, M., Ibrahim, M., Koekemoer, L.L., Brooke, B.D., Engers, H., Aseffa, A., Gebre Michael, T. and El-Hassen, I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and southwestern Ethiopia and detection of *kdr* mutation. *Parasites Vectors* 3, 40.

Balkew, M., Getachew, A., Chibsa, S., Olana, D., Reithinger, R. and Brogdon, W. (2012). Insecticide resistance: A challenge to malaria vector control in Ethiopia. *Malar. J.* 11, 139.

Bouwman, H. (2000). Malaria control and the paradox of DDT. *Afr Environ Wildlife* 8, 54-56.

Chandre, F., Dabire, K.R., Hougard, J., Djogbenou, S.L., Irish, R.S., Rowland, M. and N'Guessan, R. (2010). Field efficacy of pyrethroid treated plastic sheeting (durable lining) in combination with long lasting insecticidal nets against malaria vectors. *Parasites Vectors* 3, 65.

Corbel, V., Chabi, J., Dabiré, K.R., Etang, J., Nwane, P., Pigeon, O., Akogbeto, M. and Hougard, J. (2010). Field efficacy of a new mosaic long-lasting mosquito net (PermaNet® 3.0) against pyrethroid-resistant malaria vectors: a multi centre study in Western and Central Africa. *Malar. J.* 9, 113.

Etang, J., Chandre, F., Guillet, P. and Manga, L. (2004). Reduced bio-efficacy of permethrin EC impregnated bed nets against *Anopheles gambiae* strain with oxidase-based pyrethroid tolerance. *Malar. J.* 3, 46.

Fettene, M., Olana, D., Christian, R.N., Koekemoer, L.L., Coetzee, M. (2013). Insecticide Resistance in *Anopheles arabiensis* from Ethiopia. *Afri. Entomol.* 21, 89-94.

FMoH. (2012). Ethiopia national malaria indicator survey 2011: technical summary, federal ministry of health, Addis Ababa, Ethiopia. September 2012.

Gebremariam, N. (1988). *Malaria*. In: Zein ZA, Kloos H (eds). The ecology of health and disease in Ethiopia. Ministry of health, Addis Ababa, 1988.

Gerberg, E.J., Barnard, D.R. and Ward, R.A. (1994). *Manual for mosquito rearing and experimental techniques*. American mosquito control association, Inc.

Gillies, M.T. and Coetzee, M. (1987). A supplement to the anophelinae of Africa South of the Sahara, Johannesburg. Publications of the South African institute of medical research 1987.

Guyatt, H.L., Corlett, S.K., Robinson, T.P., Ocholas, S., Robert, S.W. (2002). Malaria prevention in highland Kenya: indoor residual house spraying vs. insecticide treated bed nets. *Trop. Med. Int. Health* 7, 298-303.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human diseases. *Annu. Rev. Entomol.* 45, 371-391.

Kawada, H., Dida, O.G., Ohashi, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Maekawa, Y., Mwatele, C., Njenga, M.S., Mwandawiro, C., Minakawa, N. and Takagi, M. (2011). Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae s.s*., *Anopheles arabiensis* and *Anopheles funestus* in Western Kenya. *PLoS One* 6, e22574.

Kelly-Hope, L., Ranson, H. and Hemingway, J. (2008). Lessons from the past: managing insecticide resistance in malaria control and eradication programs. *Lancet Infect. Dis.* 8, 387-389.

Koffi, A.A., Ahoua-Alou, P.L., Adja, A.M. and Chandre, F. (2013). Insecticide resistance status of *Anopheles gambiae s.s* population from M'Bé: a WHOPES-labelled experimental hut station, 10 years after the political crisis in Côte d'Ivoire. *Malar. J.* 12, 151.

Koudou, G.B., Ghattas, H., Essé, C., Nsanzabana, C., Rohner, F., Utzinger, J., Faragher, E.B. and Tschannen, B.A. (2010). The use of insecticide-treated nets for reducing malaria morbidity among children aged 6-59 months, in an area of high malaria transmission in central Côte d'Ivoire. *Parasites Vectors* 2, 91.

Koudou, G.B., Koffi, A.A., Malone, D. and Hemingway, J. (2011). Efficacy of permaNet® 2.0 and permaNet® 3.0 against insecticide-resistant *Anopheles gambiae* in experimental huts in Côte d'Ivoire. *Malar. J.* 10, 172.

Massebo, F., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2013). Blood meal origins and insecticide susceptibility of *Anopheles arabiensis* from Chano in South-West Ethiopia. *Parasites Vectors* 6, 44.

N'Guessan, R., Corbel, V., Akogbeto, M. and Rowland, M. (2007). Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect. Dis.* 13, 199-206.

Najera, J.A. and Zaim, M. (2001). Malaria vector control insecticides for indoor residual spraying. Available from: WHO_CDS_WHOPES_2001.3 [accessed: 21/09/2016].

Ngufor, C., N'Guessan, R., Boko, P., Odjo, A., Vigninou, E., Asidi, A., Akogbeto, M. and Rowland, M. (2011). Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant *Anopheles gambiae*: an experimental hut trial in Benin. *Malar. J.* 10, 343.

Norris, C.L. and Norris, E.D. (2011). Efficacy of long-lasting insecticidal nets in use in Macha, Zambia, against the local *Anopheles arabiensis* population. *Malar. J.* 10, 254.

Okumu, O.F., Chipwaza, B., Madumla, P.E., Mbeyela, E., Lingamba, G., Moore, J., Ntamatungro, J.A., Kavishe, R.D. and Moore, J.S. (2012). Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and long lasting insecticide nets are combined for malaria prevention. *Malar. J.* 11, 378.

Ranson, H., Abdallah, H., Badolo, A., Guelbeogo, M.W., Kerah-Hinzoumbé, C., Yangalbé-Kalnoné, E., Sagnon, N., Simard, F. and Coetzee, M. (2009). Insecticide resistance in *Anopheles gambiae*: data from the first year of a multi-country study highlight the extent of the problem. *Malar. J.* 8, 299.

Tulu, A.N. (1993). Malaria: In Kloos H and Zein ZA (eds). *The ecology of health and disease in Ethiopia.* Boulder, Westview Press.

Tungu, P., Magesa, S., Maxwell, C., Malima, R., Masue, D., Sudi, W., Myamba, J., Pigeon, O. and Rowland, M. (2010). Evaluation of permaNet® 3.0 a deltamethrin-PBO combination net against *Anopheles gambiae* and pyrethroid resistant *Culex quinquefasciatus* mosquitoes: an experimental hut trial in Tanzania. *Malar. J.* 9, 21.

Van Bortel, W., Chinh, D.V., Berkvens, D., Speybroeck, N., Trung, D.H. and Coosemans, M. (2009). Impact of insecticide-treated nets on wild pyrethroid resistant *Anopheles epiroticus* population from southern Vietnam tested in experimental huts. *Malar. J.* 8, 248.

Vannice, K.S., Brown, G.V., Kenmore, B.D., Moorthy, V.S. (2012). MALVAC 2012 Scientific forum: accelerating development of second-generation malaria vaccines. *Malar. J.* 11, 372.

WHO (1998). *Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy, and persistence of insecticides on treated surfaces.* WHO Press, World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland: World health organization; available from: WHO/CDS/CPC/MAL/98.12 [accessed: 21/09/2016]

WHO (2006). *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets.* WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland: world health organization; online available from: WHO/CDS/NTD/WHOPES/2006.3. [accessed on: 21/09/2016]

WHO (2006). *Indoor residual spraying. Use of indoor residual spraying for scaling up global malaria control and elimination Programme.* WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland: World Health Organization; Online available from: WHO/HTM/MAL/2006.1112 [accessed on: 21/09/2016]

WHO (2006). *Pesticides and their application for the control of vectors and pests of public health importance.* 6th ed. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerlan: World health organization; available from: WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf [accessed: 21/09/2016]

WHO (2007). *Long-lasting insecticidal nets for malaria prevention: World health organization manual for malaria program managers.* WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland: world health organization; online available from: LongLastingInsecticidalNetsMalaria.pdf [accessed on: 21/09/2016]

WHO (2010). C*oordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control: global malaria programme.* Geneva: world health organization.

WHO (2010). *Global report on anti-malarial drug efficacy and drug resistance: 2000–2010.* WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland: world health organization.

WHO (2012). *World Malaria Report 2012.* WHO Press, World health organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland: World health organization. Available from: 9789241564533_eng [accessed: 21/09/2016]

Yewhalaw, D., Asale, A., Tushune, K., Getachew, Y., Duchateau, L. and Speybroeck, N. (2012). Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant *Anopheles arabiensis* from southwestern Ethiopia. *Parasites Vectors* 5, 159.

Yewhalaw, D., Bortel, V.W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Am. J. Trop. Med. Hyg.* 83, 122-125.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Leen D., Tessema, D.A., Getachew, Y, Marc, C., Duchateau L. and Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PLoS One* 6, e16066.

Chapter 5

Assessing the host preference of *Anopheles arabiensis* (Diptera: Culicidae) in southwestern Ethiopia

Adapted from

Asale, A., Emana, D., Zemene, E., Alemayehu, E., Habtewold, T., Yewhalaw, D. and Duchateau, L. (2016). Assessing the host preference of *Anopheles arabiensis* (Diptera: Culicidae) using three alternative experimental setups in southwestern Ethiopia. *Journal of insect Behavior*. Under revision.

5.1 Abstract

Combining zooprophylaxis with other strategies has the potential to further strengthen vector control intervention efforts. However, such complementary approaches require a good understanding of vector host feeding preference. This study assessed host preference of *Anopheles arabiensis*, the primary vector of malaria in Ethiopia. The host preference of *Anopheles arabiensis* was assessed by comparing mosquito density in enclosure traps (Experiments 1 and 2) and blood meal source preference (Experiment 3) between humans and livestock hosts (calf, goats, donkeys, and chicken).

The density of An. arabiensis was significantly higher in the enclosure trap with calf ($P < 0.001$) as compared to the enclosure trap with human whereas, the density of An. arabiensis was significantly lower in the enclosure trap with chicken ($P = 0.002$) and goat ($P < 0.001$) as compared to the enclosure trap with human. In the second experiment, An. arabiensis density was significantly higher in the enclosure trap with donkey ($P = 004$), calf ($P < 0.001$) and goat ($P < 0.001$) as compared to the enclosure trap with human. Similarly, a significantly higher number of An. arabiensis mosquitoes fed on calf as compared to human ($P< 0.001$).

The results of all three different host preference experimental setups showed that populations of Anopheles arabiensis from Jimma area were zoophagic with respect to cattle but anthropophagic with respect to chicken. The outcomes are less apparent for the other two livestock hosts (equine and ovine). Thus, cattle could have a potential role in diverting malaria vectors away from human and thus reduce human-vector contact in vector control interventions.

Keywords: Zooprophylaxis**,** livestock host, malaria, host preference, mosquito density, *Anopheles arabiensis*, Ethiopia

5.2 Introduction

Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are pillars of malaria vector control. They target female anophelines that feed indoors. Wide-scale reduction in malaria burden has been achieved following the scale up in coverage of LLINs and IRS (WHO, 2015). However, the efficacy of LLINs and IRS is threatened by the development of physiological resistance to one or more insecticide classes (e.g., pyrethroids, organophosphates, carbamates and DDT) which are used for these tools (WHO, 2013; Mulamba et al., 2014; Toe et al., 2014; Temu et al., 2012; Yewhalaw et al., 2011; Balkew et al., 2010). Moreover, there is also growing evidence of a behavioral change in the mosquito population following repeated application of IRS and LLINs. These include biting early in the evening before people retire to bed, feeding outdoor, resting outdoor and increased preference to feed on livestock (Yohannes and Boelee, 2012; Russell et al., 2011; 2013; Killeen, 2014; Ranson et al., 2011; WHO, 2014).

An. arabiensis, a member of the gambiae complex and the main malaria vector in Ethiopia, displays plasticity in its behavior (biting time, blood meal source and resting place) depending on the environmental circumstances. For example, the resting behavior of *An. arabiensis* can be endophilic or exophilic depending on the availability of a shed (Faye et al., 1997), host location (Tirados et al., 2006) and IRS application/treated wall surfaces (Reddy et al., 2011; Padonou et al., 2012). *Anopheles arabiensis* can readily feed on a range of livestock hosts including bovine (Massebo et al., 2015; Duchemin et al., 2001; Hadis et al., 1997), ovine (Waka et al., 2005) and human (Tirados et al., 2006; Fornadel et al., 2010; Fontenille et al., 1997). The time of host-feeding varies depending on the host preference and the availability of the host indoor or outdoor but host feeding by this mosquito species is mostly concentrated in the first and last quarter of the night (Githeko et al., 1996; Taye et al., 2016; Yohannes and Boelee, 2012). This high behavioral plasticity of *An. arabiensis* makes its control difficult by currently available control tools (IRS and LLINs) which target indoor feeding and resting mosquitoes.

Thus, innovative vector control tools that target vectors when resting and/or feeding outdoors should be developed in order to sustain the gains achieved with LLINs and IRS. One such potential vector control tool is zooprophylaxis (the use of animals for diversion of blood seeking mosquitoes away from human). The role of zooprophylaxis has not been advocated much in the past due to the controversial reports on its feasibility and efficacy. There are several reports supporting implementation of zooprophylaxis in malaria control (Muriu et al. 2008; Mahande et al., 2007; Bultery et al., 2009) but equally there are studies reporting increased

malaria transmission due to the presence of livestock (i.e., zoopotentiation) (Temu et al., 2012; Tirados et al., 2011; Palsson et al., 2004; Githinji et al., 2009). Facing the current challenge of controlling residual malaria transmission, the WHO has recently recommended assessing other strategies including the use of topical or systemic insecticides for livestock treatment that could kill mosquitoes during or after feeding (WHO, 2014).

In Ethiopia, *An. arabiensis* is the primary vector of malaria (Tulu, 1993). Its peak biting time is early in the evening and last quarter of the night (Yohannes and Boelee, 2012; Taye et al., 2016). It is equally exophagic and endophagic (Taye et al., 2016). It feeds readily on both livestock hosts such as bovine and ovine (i.e., zoophagic) (Habtewold et al., 2001; Seyoum et al., 2002; Animut et al., 2013; Massebo et al., 2015) and humans (i.e., anthropophagic) (Tirados et al., 2006; Tirados et al., 2011). However, it has not been investigated whether the reported zoophagic behavior is due to accessibility of the host or due to an inherent preference of the mosquitoes. Thus, implementing zooprophylaxis requires quantifying the preference of the local population of *An. arabiensis* to different livestock hosts. It is essential that mosquitoes are not only at first more attracted to a particular livestock host, but that they also feed on it. This quantitative and comprehensive information on host preference is instrumental to plan and implement zooprophylaxis as part of vector control intervention. Thus, the primary objective of this study was to assess the host preference of populations of *An. arabiensis* with respect to livestock hosts (bovine, ovine, equine and chicken) in southwestern Ethiopia.

5.3 Materials and methods

5.3.1 Study setting

The study was conducted in Omo Nada district, Jimma zone, Oromia Regional State, southwestern Ethiopia. The study area located between latitudes $07^0$42’37 N-$07^0$53’50 N and longitudes $037^0$11’22 E- $037^0$20’36 E at an altitude of 1670-1784 masl. The study area is characterized by a black cotton soil with a thin top layer of humus and ever green plants. The area has a dry and warm climate with a mean annual temperature of 19.2 ^{0}C and annual rainfall that varies from 1300 mm 1800 mm. The rainfall pattern of the area is similar to other parts of Ethiopia with the long rainy season starting in June and extending up to September while the short rainy season begins in March and extends to April/May. The major livestock in the study area are cattle followed by poultry and goats, with human to livestock ratios of 1.15:1, 1.50:1, and 2.2:1 respectively (Musin, 2010).

5.3.2 Study design

5.3.2.1 Establishment of experimental huts

Four experimental huts (6m x 3m) were constructed from cement brick with roofs made of corrugated iron sheet and ceilings from white sheets following the WHO guideline for the West African hut type (WHO 2005). All the windows were with slits. In each experimental hut there were two enclosure traps (2m x 1.5m x 2m) made of white sheet and iron frame, erected side by side with one-meter space between them (Habtewold et al. 2004). Each enclosure trap has a 30 cm opening left at its bottom (Figure 5.1). A cattle crush was made to keep the livestock host in a fixed position while a rectangular metal pan was used to collect urine and droppings of the livestock hosts (Figure 5.1). The livestock hosts used in this study were: a Zebu calf, a donkey and a goat of approximately 1.5 years old and approximately weighing 150kg, 140kg and 60kg respectively and a cock approximately 1-year-old weighing approximately 3kg (according to local farmer's information).

5.3.2.2. Experiment I: comparing host preference based on mosquito density entering the experimental huts from the field

The first experimental setup was conducted from June to July 2015. A Latin square design was used to compare the four treatments (donkey/human, calf/human, goat/human and chicken/human) using hut and night as blocking factor, i.e., each treatment appeared once in each hut and in each night. After one night of measurement, the hut was aerated for 24 hours, so that a complete run of one Latin square took 8 days. The Latin square was repeated 4 times resulting in a total of 16 nights (rotations) with measurements. Note that human volunteers were linked to the same hut throughout the study whereas animals were subjected to rotate every other day. Thus, a total of 16 collection nights were made for each treatment. During the experiment (collection night), both animal hosts and human volunteers entered and exited the experimental hut at 19:00 and 07:00 hours respectively. In this set up, mosquitoes from the field were allowed to enter into the hut through window slits, and once the mosquitoes were in, they had a choice to enter into one of the two enclosure traps through a 30 cm opening left at the bottom of each of the enclosure trap.

A

B

C

Figure 5.1 Experimental set up of the study A. Field experimental huts, B. Enclosure trap showing bottom slit, C. Animal crush and rectangular metal pan (floor stand) used for dung and urine collection.

Mosquitoes were collected early in the morning (06:00-07:00 hours) from inside each enclosure trap by trained volunteers (the sleepers themselves). At 6:00 each morning, the human volunteers remained within the enclosure trap and then unrolled the sides to the ground, after which they proceeded to collect mosquitoes from the human enclosure trap. Then they

exited the human enclosure trap and first unrolled the side of animal enclosure trap to the ground. Then they collected mosquitoes resting in the corridor (i.e. the interior space of the experimental hut including floor, wall and ceiling of the hut and the exterior part of the enclosure trap) and entered in to animal trap by slowly unfolding one side of the trap and immediately unrolling back. The animal remained inside the trap until the end of collection. Then mosquitoes resting inside the animal trap were collected carefully using mouth aspirators. The collected mosquitoes were then sorted as alive, dead, unfed, fed, half gravid or gravid and were identified morphologically to species level using taxonomic key (Gillies and Coetzee, 1987). *An. gambiae* s.l. were not further molecularly identified in to sibling species, but we presume that the *An. gambiae* s.l. are overwhelmingly belong to *An. arabiensis* as it was reported by Yewhalaw et al. (2010) from the same area. All alive mosquitoes were killed in -20^0C freezer, kept individually, in an eppendorf tube (1.5ml) containing cotton and silica gel. The eppendorf tubes were then stored inside plastic box. Both livestock and human volunteers were kept out of the hut during the daytime and in non-collection nights; the hut was cleaned, left open for aeration. This experiment was run from June to July 2015. Host preference was assessed by the difference between mean mosquito density attracted to livestock host and human volunteer (both fed and unfed mosquitoes collected inside each enclosure trap were considered as mosquitoes attracted to a given host host).

5.3.2.3 Experiment II: Comparing host preference based on mosquito density attracted to livestock host and humans using release-recapture method

The second experimental setup, conducted from July to August 2015, was similar to experiment one except that mosquitoes were released into the experimental huts that remained closed once livestock and human volunteers were in to deny possible escape of mosquitoes from the hut. Anopheline larvae were collected by dipping from a range of breeding sites and reared to adults in Sekoru Field Vector Biology Laboratory, Jimma University under standard conditions (temperature 25 ± 2^{o}C, relative humidity 80 ± 4%) (Gerberg, 1970; Looker and Taylor-Robinson, 2013). Anopheline mosquito larvae collected from the field were visually identified on site from other sympatrically existing culicine and aedine larvae by their orientation, presence/absence of respiratory siphon, size of head region and body appearance (Williams and Pinto, 2012). Four cups labelled with hut number and dates were prepared ahead of the experiment. Fifty, 3 to 5 days old adult female mosquitoes belonging to *An. gambiae* s.l, presumably *An. arabiensis* (Yewhalaw et al., 2010) were aspirated from a cage and transferred to each of the four cups. A known number (range = 50-55) of mosquitoes were released inside

each hut mid-way between the two enclosure traps. Each night one livestock host was tethered in of the enclosure traps and a human volunteer was allowed to sleep inside the second enclosure trap between 19:00 to 6:00 hours. The mosquitoes could enter into one of the enclosure traps either with livestock host or human through an opening (30 cm) left by rolling up one side of the enclosure trap above the ground. Mosquitoes collected from each enclosure trap between 06:00 to 07:00 hours by the same human volunteers who slept in the enclosure trap. At 6:00 each morning, the human volunteers remained within the enclosure trap and then unrolled the sides to the ground, after which they proceeded to collect mosquitoes from the human enclosure trap. Then they exited the human enclosure trap and first unrolled the side of animal enclosure trap to the ground. Then they collected mosquitoes resting in the corridor (defined in experiment 1) and entered in to animal trap by slowly unfolding one side of the trap and immediately unrolling back. The animal remained inside the trap until the end of collection. Then mosquitoes resting inside the animal trap were collected carefully using mouth aspirators. The collected mosquitoes were then transferred to labeled paper cups. Mosquitoes were then sorted as alive, dead, unfed and fed. All alive mosquitoes were killed in -20 freezer kept individually, in an eppendorf tube (1.5ml), and stored in a plastic box over desiccant silica gel. Experimental huts were aerated for 24 hours following each collection night. Study design and treatment combinations were implemented in the same way as experiment one.

5.3.2.4 Experiment III: Comparing host preference based on blood meal source

The third experiment was conducted from September to October 2015. In this experiment, enclosure traps were removed from each experimental hut and each livestock host was tethered inside an experimental hut and a human volunteer was also allowed to sleep in the same experimental hut next to the animal in such a way that the mosquitoes could choose freely between the two hosts. Anopheline mosquito larvae were collected from the field and reared to adults under standard conditions (temperature 25 ± 2^{o}C, relative humidity 80 ± 4%) (the larval identification procedure is described before). Fifty to fifty-five, 3 to 5 days old adult female mosquitoes belonging to *An. gambiae* s.l, presumably *An. arabiensis* (Yewhalaw et al. 2010) were aspirated from a stock cage, transferred to each cup labeled by date and hut identification number. Then fifty-five mosquitoes were released inside each hut after which doors and windows were closed by the volunteers assigned to sleep inside the huts. Mosquitoes were retrieved between 6:00 to 07:00 hours by trained volunteers (sleepers) from the wall, floor, and ceiling using mouth aspirators. The collected mosquitoes were then transferred to labeled paper cups. Dead mosquitoes collected from the floor were counted and recorded on-spot before

transferring them to separate paper cups. All retrieved live mosquitoes were killed in -20^{0}C freezer for 10 to 15 min and sorted as unfed or fed. Each mosquito was kept individually in an eppendorf tube (1.5ml) containing silica gel and cotton, labeled and stored in a plastic box. Mosquitoes were declared lost after a 30-minute search. Following each collection night, each hut was left open for a period of 24 hours to be aerated. A 4 x 4 Latin square design was employed as before to randomize the treatments (human/cattle, human/donkey, human/goat and human/chicken) using hut and night as blocking factor and the Latin square design was repeated 4 times as before. The blood meal source of mosquitoes collected inside each hut was determined using direct blood meal ELISA.

5.3.2.5 Determination of mosquito blood meal source

Blood meal source was detected using direct ELISA (Beier et al., 1988). The abdomen of each fed female mosquito was homogenized in 50 µl of phosphate buffer saline (PBS, PH 7.4) using a pestle in 1.5 ml grinding tube and final volume brought to 200 µL with PBS buffer. Fifty microliter of the diluted sample was added into wells of micro ELISA plate; wells were covered and incubated at room temperature for 3 hours. The homogenate was discarded and the plate was washed thrice with 200 µl of PBS–Tween20. Fifty micro liter peroxidase conjugate antibody of human, equine, chicken, ovine and 50 µl phosphatase conjugate antibody of bovine (SIGMA-ALDRICH) was added to the respective wells in the micro ELISA plate and incubated for one hour at room temperature. In this experiment, we used horse antibody to identify donkey host following the method used by (Lemasson et al., 1997). After one hour, wells were washed 3 times with PBS–Tween 20. Then, 100 µl ABTS peroxidase substrate solution was added to each coated micro ELISA plate for donkey, human, goat and chicken. The results were read both visually and using the microplate absorbance reader at a wavelength of 405 after 30 minutes. In this assay, the double testing system was employed for humans and bovine. Thus, plates read for human antibody were washed thrice with 200 µl of PBS–Tween20 and 100 µl of pNPP phosphatase was added to each well. Finally, the results were read both visually and using the microplate absorbance reader at a wavelength of 405 after 30 minutes. Blood samples collected from jugular vein puncture of vertebrates using EDTA coated vacuum tubes were used as positive control. Unfed laboratory reared female mosquitoes were used as negative control in the assay.

5.3.3 Data analysis

Logistic regression analysis was used to assess the difference in preference between the different livestock hosts and the human volunteer. The response was the number of mosquitoes preferring the livestock host as compared to the total number of mosquitoes that made a choice, i.e., the total number of mosquitoes in the two enclosure traps in experiments 1 and 2, and the total number of mosquitoes with a blood meal from one host in experiment 3. The logistic regression model contained the livestock host as categorical fixed effect and hut and night were added to the model as adjusting factors. The results are summarized as odds ratios with their 95% confidence interval, but also as the percentage preference of the livestock host, with a value of 50% signifying no preference, above 50% a preference for the livestock host and below 50% a preference for the human volunteer. The human blood index was calculated as the proportion of specimens containing human blood. All analyses were done using SAS software package version 9.4 (SAS Institute Inc., Cary, NC, USA).

5.3.4 Ethical consideration

The study protocol was reviewed and approved by the research and ethics committee of Jimma University, Ethiopia. All human volunteers were trained for field mosquito collection and volunteers were provided with mefloquine as chemoprophylaxis as per the national malaria diagnosis and treatment guideline and each volunteer was monitored every other day for fever. The volunteers were not vaccinated against yellow fever as there were no previous reports of yellow fever infection in the study area.

5.4 Results

5.4.1 Determination of host preference based on mosquitoes entering the experimental hut from the field

In the first experimental setup, a total of 1,825 mosquitoes were collected from the 4 experimental huts over the four weeks' collection period. The specimens were of 776 (43%) were *An. arabiensis,* 115 (6%) were other Anopheline species and 934 (51%) were Culex spp. The *An. arabiensis* density was significantly higher in the enclosure trap with calf as compared to human (OR=2.38, $P < 0.001$), whereas the density was significantly lower in the enclosure trap with chicken (OR=0.40, $P = 0.002$) and goat (OR=0.12, $P < 0.0012$) as compared to

enclosure trap with human. There was no significant difference in density between enclosure traps with human and donkey OR=0.85, P = 0.486) (Figure 5.2). The host preference was 70.41 % for calf, but below 50% and equal to 46.03%; 28.38% and 11.10% for donkey, chicken and goat respectively.

5.4.2 Determination of mosquito host preference based on release-recapture method

Overall, 3,115 *An. arabiensis* were retrieved over a period of 16 collection nights from all enclosure traps set inside the four experimental huts which gave a recapture rate of 97 %. Of these, 1127 (36%) were fed and 1988 (64%) were unfed. The *An. arabiensis* density was significantly higher in the enclosure trap with donkey (OR=1.29, P = 0.005), calf (OR=1.56, P < 0.001) and goat (OR=1.40, P < 0.001) as compared to the enclosure trap with human. However, the density was significantly lower in the enclosure trap with chicken (OR=0.45, P < 0.001) as compared to the enclosure trap with human (Figure 5.2). The host preference was in favor of the livestock host for donkey, calf and goat with preference resp. equal to 56.39%, 61.01% and 58.39% but well below 50% and equal to 30.95% for chicken.

5.4.3 Determination of mosquito host preference based on blood meal source

A total of 2,237 *An. arabiensis* were retrieved over all collection nights from all enclosure traps set inside the experimental huts which gave a recapture rate of 70 %. Of these 637 (28%) were fed and 1600 (72%) were unfed. A significantly higher number of *An. arabiensis* mosquitoes fed on calf compared to human (OR=3.16, P;< 0.001) whereas a significantly lower number of *An. arabiensis* mosquitoes fed on donkey (OR= 0.29, P < 0.001), chicken (OR=0.35, P = 0.003) and goat (OR=0.38, P = 0.019) as compared to human. The host preference was 75.97% for calf, but below 50% and equal to 22.67%; 25.76% and 27.38% for donkey, chicken and goat respectively (Figure 5.2). The human blood index for treatment one (Bovine vs human), treatment two (Equine vs human), treatment three (Ovine vs human) and in treatment four (Chicken vs human) was 36.6%, 65.6%, 52.9% and 65.4% respectively.

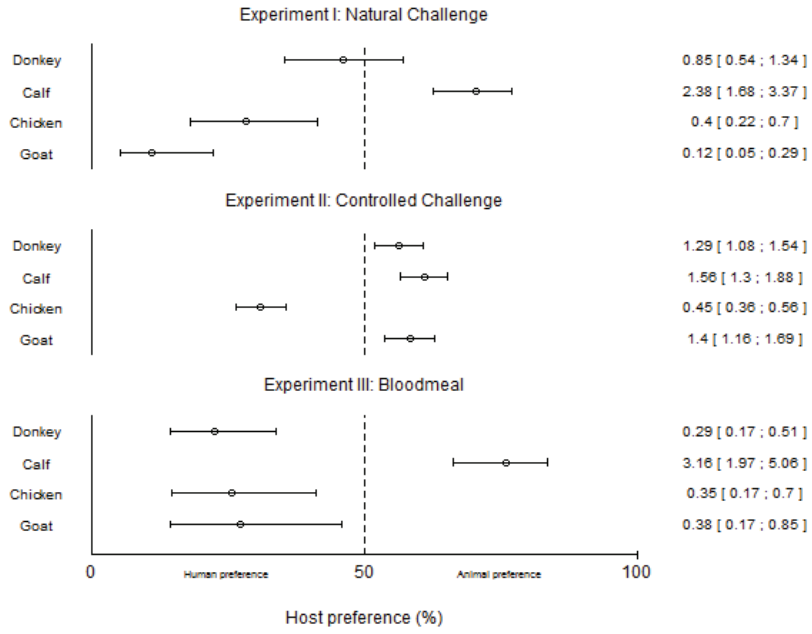

Figure 5.2 Host preference for four livestock hosts as compared to human volunteers in the three different experimental setups. The 95% confidence interval of the preference of the livestock host as compared to human is depicted by the horizontal bars. The dashed vertical line at 50% corresponds to no preference. The right side column corresponds to the odds ratio with 95% confidence interval between brackets.

5.5 Discussion

The world health organization currently recommends different novel vector control tools including topical and systemic application of insecticides on livestock that kill mosquitoes during or after feeding thereby, reducing adult vector densities and/or transmission rate (WHO, 2014). In that way, a possibly infectious bite can be prevented and the mosquito cannot acquire an infection while feeding on these animals as the plasmodium parasite does not develop in these animals. However, prior knowledge of the host preference behavior of vector species in a specific locality is essential to implement such interventions.

In this study, the mosquito host preference was determined using three different experimental setups. In all three experiments, *An. arabiensis* showed preference to feed on cattle to human. In contrast, *An. arabiensis* preferred to feed on human (i.e. anthropophagic) as compared to chicken in all of three experiments. The outcome is less straightforward for the other two livestock hosts (ovine and equine). Preference for both donkey and goats was as compared to human was observed in the controlled challenge experiment, whereas *An. arabiensis* preferred to feed on human (i.e. anthropophagic) as compared to donkey and goat in the blood meal experiment.

Preference of *An. arabiensis* to feed on cattle is well documented. Blood meal analysis of indoor/outdoor resting mosquitoes from Ethiopia, where livestock stays mostly in sheds separated from the human quarters, showed a lower human blood index (HBI) value (Massebo et al., 2015; Habtewold et al., 2001). Even in houses where a small number of livestock are kept together with people (i.e., in mixed dwellings) mosquitoes tend to either feed on cattle or take blood meals evenly (Animut et al., 2013; Haddis et al., 1997). The zoophilic behavior of *An. arabiensis* is also supported by findings from other east African countries including Tanzania (Mahande et al., 2007) and Kenya (Kaburi et al., 2009), where lower HBI was recorded in households with cattle as compared to households without cattle. Moreover, a significantly higher number of *An. arabiensis* mosquitoes were collected from cattle enclosure traps as compared to traps containing human volunteer only using odor baited entry traps in Tanzania (Mahande et al., 2007) and Madagascar (Duchemin et al., 2001). However, host preference in *An. arabiensis* may vary from place to place, type of livestock host next to human, availability and accessibility. For instance, Tirados et al. (2006) used a similar study design (except that in the current study enclosure traps were placed inside experimental huts), to compare mosquito density between the enclosure traps and to determine the blood meal source. They found that the human-baited trap caught about five times more *An. arabiensis* mosquitoes

than the cattle-baited trap and HBI ranged between 46-66%. A recent study from western Ethiopia by Jaleta et al. (2016) documented that *An. arabiensis* avoids cattle upon entering the house and mainly prefers to feed on human regardless of the availability of different livestock hosts. Anthropophilic behavior of *An. arabiensis* was also reported from Zambia by comparing mosquito density from human landing catches (HLC), cattle baited traps and analysis of HBI (Fornadel et al., 2010; Kent et al., 2007).

In our study, populations of *An. arabiensis* preferred to bite human when allowed to choose between chicken and human. This could be either due to the fact that chickens are less suitable to feed on because their body is covered with feathers or it could be due to some volatile substance emitted from their body which repels the approaching malaria vector mosquitoes. Jaleta et al. (2016) recently confirmed the later.

In our study mosquito preference to feed on donkey lacked consistency The lack of consistency could be due to the defensive behavior of the animal. Only a mild zooprohylactic effect of donkey was documented in Burkina Faso (Yamamoto et al., 2009). Similarly, significantly lower mosquitoes fed on goat (experiments 1 and 3) as compared to the density of fed mosquitoes quantified from human enclosure trap. The feeding pattern however, reversed in experiment 2 with significantly higher mosquitoes fed on goat compared to the density of mosquitoes recorded in human enclosure trap. The populations of *An. arabiensis* tend to feed on goat only in situations where other larger domestic animals are not readily available (Waka et al., 2005) and they thus have low mosquito diversion effect in the presence of other domestic animals such as cattle (Bulterys et al., 2009; Kent et al., 2007; Mahande et al., 2007).

The double screening system (i.e. the enclosure trap set inside the experimental hut) we used in the first experiment might have subjected mosquitos to a prolonged time of finding a host, which could reduce the number of mosquitoes trapped per night. In the blood meal source experiment, relatively few mosquitoes out of the total recovered, were fed (28%). This could be partly attributed to the defensive behavior of the host species. Moreover, only visually identified fed mosquitoes were tested for host choice and this might have led to an underestimation of the proportion of fed mosquitoes since some partially fed mosquitoes could be identified as unfed (Das et al., 2015).

Similar studies conducted to assess the host preference of *An. arabiensis* using release and recapture methods in experimental huts reported retention rate and resting behavior but not the proportion of fed mosquitoes (Mahande et al., 2007). It has been suggested that host preference, distance between livestock and humans are important factors in implementing zooprophylaxis (Donnelly et al., 2015). This important aspect was not assessed as the scope of this study was

limited to host preference, but should be investigated further in the context of a zooprophylactic vector control approach.

5.6 Conclusion

In conclusion, the *An. arabiensis* population in the Jimma region is zoophagic with respect to cattle but anthropophagic with respect to chicken. The outcome is less apparent for the other two livestock hosts (equine and ovine). The fact that cattle may play a potential role as a barrier or effectively divert malaria vectors from human to livestock hosts was evidenced in this study. The information from the current study could be used in mosquito population models to predict the probability of successful use of zooprophylaxis in malaria vector control. Other factors, such as the use of insecticides on the livestock host and the optimal distance between the livestock host and the human need to be further investigated.

Acknowledgements

Jimma University VLIR-IUC program is acknowledged for logistic and financial support. The authors are grateful to Asendebo Vector Biology Laboratory staff members for their excellent technical support.

5.7 References

Animut, A., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2013). Blood meal sources and entomological inoculation rates of Anophelines along a highland altitudinal transect in south-central Ethiopia. *Malar J* 12:76.

Balkew, M., Ibrahim, M., Koekemoer, L.L., Brooke, B.D., Engers, H., Aseffa, A., Gebre Michael, T. and El-Hassen, I. (2010). Insecticide resistance in *Anopheles arabiensis* (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of *kdr* mutation. *Parasite Vectors* 3:40.

Beier, J.C., Perkings, P.V., Wirtz, R.A., Koros, J., Diggs, D., Garganii, T.P. and Koech, D.K. (1988). Blood meal identification by direct enzyme linked immunosorbent assay (ELISA), tested on Anopheles (Diptera: Culicidae) in Kenya. *J Med Entomol* 25: 9-16.

Bulterys, P.L., Mharakurwa, S. and Thuma, P.E. (2009). Cattle, other domestic animal ownership, and distance between dwelling structures are associated with reduced risk of recurrent *Plasmodium falciparum* infection in southern Zambia. *Trop Med Int Health* 14: 522-528.

Das, S., Henning, T., Simubali, L., Hamapumbu, H., Nzira, L., Mamini, E., Makuwaza, A., Muleba, M., Norris, D., Stevenson, J. and Southern Africa ICEMR Team (2015). Underestimation of foraging behaviour by standard field methods in malaria vector mosquitoes in southern Africa. *Malar J* 14:12.

Donnelly, B., Berrang‐Ford, L., Ross, A.N. and Michel, P. (2015). A systematic, realist review of zooprophylaxis for malaria control. *Malar J* 14:313.

Duchemin, B.J., Leong, P., Tsy, M.J., Rabarison, P., Roux, J., Coluzzi, M. and Costantini, C. (2001). Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odor-baited entry traps. *Med Vet Entomol* 15: 50-57.

Faye, O., Konate, L., Mouchet, J., Fontenille, D., Sy, N., Hebrard, G.and Herve, J.P. (1997). Indoor resting by outdoor biting females of Anopheles gambiae complex (Diptera: Culicidae) in the Sahel of northern Senegal. *J Med Vet Entomol* 34: 285-289.

Fontenille, D., Lochouarn, L., Diatta, M., Sokhna, C., Dia, I., Diagne, N., Lemasson, J.J., Ba, K., Tall, A., Rogier, C. Trape, J.F. (1997). Four years entomological study of the transmission of seasonal malaria in Senegal and the bionomics of Anopheles gambiae and *An. arabiensis*. *Trans R Soc Trop Med Hyg* 91: 647-652.

Fornadel, C.M., Norris, L.C., Glass, G.E. and Norri, D.E. (2010). Analysis of Anopheles arabiensis blood feeding behavior in Southern Zambia during the two years after introduction of insecticide-treated bed nets. *Am J Trop Med Hyg* 83: 848-853.

Gerberg, E.J. (1970). Manual for mosquito rearing and experimental techniques. *AMCA Bulletin* 5:10-124.

Gillies, T. and Coetzee, M. (1987). A supplement to The Anopheline of Africa south of the Sahara, Johannesburg: The South African Institute for Medical Research No, 55.

Githeko, A.K., Adungo, N.I., Karanja, D.M., Hawley, W.A., Vulule, J.M., Seroney, I.K., Ofulla, A.V.O., Atieli, F.K., Ondijo, S.O., Genga, I.O., Odada, P.K., Situbi, .P.A and Oloo, J.A. (1996). Some observations on the biting behaviour of *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles funestus* and their implications for malaria control. *Exp Parasitol* 82: 306-315.

Githinji, S., Herbst, S. and Kistemann, T. (2009). The Human Ecology of Malaria in a Highland Region of South-West Kenya. *Methods Infec Med* 48: 451-453.

Habtewold, T., Prior, A.T., Torr, J.S. and Gibson, G. (2004). Could insecticide-treated cattle reduce Afrotropical malaria transmission? Effects of deltamethrin-treated Zebu on *Anopheles arabiensis* behavior and survival in Ethiopia. *Med Vet Entomol* 18, 408–417.

Habtewold, T., Walker, A.R., Curtis, C.F., Osir, E.O. and Thapa, N. (2001). The feeding behaviour and *Plasmodium* infection of *Anopheles* mosquitoes in southern Ethiopia in relation to use of insecticide-treated livestock for malaria control. *Trans R Soc Trop Med Hyg* 95: 584-586.

Hadis, M., Lulu, M., Makonnen, Y. and Asfaw, T. (1997). Host choice by indoor-resting Anopheles arabiensis in Ethiopia. *Trans R Soc Trop Med Hyg* 91: 376-378.

Jaleta, T.K., Hill, R.S., Birgersson, G., Tekie, H. and Ignell, R. (2016). Chicken volatiles repel host‐seeking malaria mosquitoes. *Malar J* 15:354.

Kaburi, J.C., Githuto, J.N., Muthami, L., Ngure, .P.k, Mueke, J.M. and Mwandawiro, C.S. (2009). Effects of long-lasting insecticidal nets and zooprophylaxis on mosquito feeding behaviour and density in Mwea, Central Kenya. *J Vector Borne Dis* 46: 184-190.

Kent, J.R., Thuma, E.P., Mharakurwa, S. and Norris, E.D. (2007). Seasonality, blood feeding behavior and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *Am J Trop Med Hyg* 76(2): 267–274.

Killeen, F.G. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar J* 13:330.

Lemasson, J.J., Fontenille, D., Lochouarn, L., Dia, I., Simard, F., Ba, K., Diop, A., Diatta, M. and Molez, J. (1997). Comparison of behavior and vector efficiency of *Anopheles gambiae* and *An. Arabiensis* (Diptera: Culicidae) in Barkedji, a Sahelian area of Senegal. *J Med Entomol 34:* 396 – 403.

Looker, M., and Taylor-Robinson, A.W. (2013). Mosquitoes and Parasites. IN: Kirsten Moll, Akira Kaneko, Arthur Scherf and Mats Wahlgren (Eds). *Methods in Malaria Research*. 6th Ed. Manassas, VA, USA: MR4/ATCC .

Mahande, A., Mosha, F., Mahande, J. and Kweka, E. (2007). Feeding and resting behavior of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malar J* 6:100.

Massebo, F., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2015). Zoophagic behavior of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasite Vectors* 8:645.

Mulamba, C., Riveron, J.M., Ibrahim, S.S., Irving, H., Barnes, K.G. and Mukwaya, L.G. (2014). Widespread pyrethroid and DDT resistance in the major malaria vector Anopheles funestus in East Africa is driven by metabolic resistance mechanisms. *PLoS One* 9:e110058.

Muriu, M.S., Muturi, J.E., Shililu, I.J., Mbogo, M.C., Mwangangi, M.J., Jacob, G.B., Irungu, W.L., Mukabana, W.R., Githure, I.J. and Novak, J.R. (2008). Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya. *Malar J* 7:43.

Musin, K. (2010). *Studies on Anopheles Mosquitoes Host Preference and Malaria Transmission Intensity Using Immunological Diagnostic Methods in Gilgel-Gibe Dam Area, Southwestern Ethiopia.* A Thesis Submitted to School of Graduate Studies, Ababa University in partial fulfillment of the Degree of Masters of Science in Biotechnology. Addis Ababa University.

Padonou, G.G., Gbedjissi, G., Yadouleton, A., Azondekon, R., Razack, O., Oussou, O., Gnanguenon, V., Rock, A., Sezonlin, M. and Akogbeto, M. (2012). Decreased proportions of indoor feeding and endophily in Anopheles gambiae s.l. populations following the indoor residual spraying and insecticide-treated net interventions in Benin (West Africa). *Parasite Vectors.* 5: 262-272.

Palsson, K., Jaenson, T.G.T., Dias, F., Laugen, A.T. and Bjorkman, A. (2004). Endophilic Anopheles mosquitoes in Guinea Bissau, West Africa, in relation to human housing conditions. *J Med Entomol* 41: 746-752.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z. and Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol* 27:91-98.

Reddy, M.R., Overgaard, H.J., Abaga, S., Reddy, V.P., Caccone, A., Kiszewski, A.E. and Slotman, M.A. (2011). Outdoor host seeking behavior of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar J.* 10: 184.

Russell, L.T., Beebe, W.N., Cooper, D.R., Lobo, F.N. and Burkot, R.T. (2013). Successful malaria elimination strategies require interventions that target changing vector behaviors. *Malar J* 12:56.

Russell, L.T., Govella, J.N., Azizi, S., Drakeley, J.C., Kachur, P.S. and Killeen, F.G. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J*, 10:80.

Seyoum, A., Balcha, F., Balkew, M., Ali, A. and Geberemichael, T. (2002). Impact of cattle keeping on human biting rate of Anopheline mosquitoes and malaria transmission around Ziway, Ethiopia. *East Afr Med J* 79: 486-490.

Taye, A., Hadisa, M., Adugnaa, N., Tilahuna, D. and Wirtzb, R.A. (2006). Biting behavior and Plasmodium infection rates of Anopheles arabiensis from Sille, Ethiopia. *Acta Tropica* 97: 50-54.

Taye, B., Lelisa, K., Emana, D., Asale, A. and Yewhalaw, D. (2016). Seasonal Dynamics, Longevity, and Biting Activity of Anopheline Mosquitoes in Southwestern Ethiopia. *J Insect Sci* 16(1): 6; 1–7.

Temu, E.A., Coleman, M., Abilio, A.P. and Kleinschmidt, I. (2012). High prevalence of malaria in Zambezia, Mozambique: The protective effect of IRS versus increased risks due to pig-keeping and house construction. *PloS O*NE 7:2: e31409.

Tirados, I., Costantini, C., Gibson, G. and Torr, S.J. (2006). Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med Vet Entomol* 20:4: 425-437.

Tirados, I., Gibson, G. Young, S. and Torr, S.J. (2011). Are herders protected by their herds? An experimental analysis of zooprophylaxis against the malaria vector *Anopheles arabiensis*. *Malar J* 10:68.

Toe, K.H., Jones, C.M., N'Fale, S., Ismail, H.M., Dabire, R.K., Ranson, H. (2014). Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. *Emerg Infect Diseases* 20:1691–6.

Tulu, A.N. (1993) *Malaria.*In: Kloos H. and Zein A.Z. (Eds.), The Ecology of Health and Disease in Ethiopia, Westerview Press, Boulder pp. 341–352.

Waka, M., Hopkins, R.J., Akinpelu, O. and Curtis, C. (2005). Transmission of malaria in the Tesseney area of Eritrea: parasite prevalence in children, and vector density, host preferences, and sporozoite rate. *J Vector Ecol* 30:27–31.

WHO, (1982). Manual on environmental management for mosquito control with special emphasis on malaria vectors. World Health Organization. Geneva, Switzerland.

WHO, (2005). World Health Organization Guidelines for Laboratory and Field testing of Long-Lasting Insecticidal mosquito nets. Geneva. [Online] Available from http://apps.who.int/iris/bitstream/10665/69007/1/WHO_CDS_WHOPES_GCDPP_2005.11.pdf [accessed on 1/16/2016].

WHO, (2013). World Malaria Report. World Health Organization. Geneva. [Online] Available from http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/ [accessed: 04/04/2016].

WHO, 2014: Control of residual malaria parasite transmission: guidance note. [Online] available from WHO/HTM/GMP/MPAC/2014.5 [accessed at 07/25/2016].

WHO, (2015). World Malaria Report. World Health Organization. Geneva. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed: 04/04/2016].

Williams, J. and Pinto, J. (2012). *Training Manual on Malaria Entomology: For Entomology and Vector Control Technicians (Basic Level)*. United States Agency for International Development. [Online]. Available from http://www.rollbackmalaria.org/files/files/partnership/wg/wg_itn/docs/ws8/TrainingManualOnMalariaEntomology-en.pdf [accessed: 2/27/16].

Yamamoto, S.S., Louis, V.R., Sie, A. and Sauerborn, R. (2009). The effects of zooprophylaxis and other mosquito control measures against malaria in Nouna, Burkina Faso. *Malar J* 8: 283 doi:10.1186/1475-2875-8-283.

Yewhalaw, D., Van Bortel W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. (2010). First evidence of high knockdown resistance frequency in Anopheles arabiensis (Diptera: Culicidae) from Ethiopia. *Am J Trop Med Hyg* 83:122–125.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Denis, L. (2011). Multiple Insecticide Resistance: An Impediment to Insecticide-Based Malaria Vector Control Program. *PloS ONE* 6:1 e16066.

Yohannes, M. and Boelee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, Anopheles arabiensis, and challenges for its control in Ethiopia. *Med Vet Entomol* 26: 103-105.

Chapter 6

Additive effect of repellent and zooprophylaxis in malaria vector control in southwestern Ethiopia

Adapted from

Asale, A., Duchateau, L., Zemene, E., Emana, D., Alemayehu, E., Eba, K., Habtewold, T., Tefera, M., Tushune, K. and Yewhalaw, D. (2016). Additive effect of repellent and zooprophylaxis in malaria vector control in southwestern Ethiopia. Submitted to Tropical Medicine Health.

6.1 Abstract

The effect of the simultaneous use of a repellent as a pushing factor and livestock as a pulling factor in order to prevent potentially infectious bites of Anopheles mosquitoes on humans has not been studied in much detail. In this study the efficacy of Buzz off (petroleum jelly, essential oil blend), Mozigone (plant derived essential oil blend) and DEET, a standard repellent, were assessed using arm-in-cage experiments and semi-field setups using human volunteers with repellent and a calf as pulling factor.

Arm-in-cage repellent testing assays were conducted using a laboratory established reference colony and wild populations of *Anopheles arabiensis* raised from field collected larvae. In the semi-field setup, the efficacy of each repellent was evaluated by comparing mosquito density in enclosure traps containing human volunteers with or without repellent (Experiment 2) and by comparing mosquito density in enclosure traps of human with repellent and calf (Experiment 3).

The median complete protection time for Buzz off, Mozigone, and DEET using wild populations of Anopheles arabiensis was 3, 61 and 302 minutes respectively. Significantly higher mosquito density was recorded in enclosure traps without repellent as compared to enclosure traps containing human volunteers used Mozigone (mean difference = 15.25; $p < 0.001$), Buzz off (mean difference = 6.25; $P = 0.045$) and DEET (mean difference = 9.75; $P = 0.008$). Similarly, significantly higher mosquito density was recorded from enclosure traps containing calf as compared to Mozigone (mean difference = 11.75; $P = 0.027$) and DEET (mean difference = 18.75; $P = 0.004$), but not for Buzz off.

Mozigone provided relatively better protection as compared to Buzz off but its bio-prospective aspects should be further examined in field studies. DEET performs substantially better in all tests in comparison with the two other repellents, Mozigone and Buzz off.

Keywords: *Anopheles arabiensis*, repellent, Buzz off, Mozigone, DEET, Ethiopia

6.2. Introduction

Long-Lasting Insecticidal Nets (LLINs) provide the primary personal protection, particularly in Sub-Saharan Africa. According to the World Health Organization, 663 million malaria cases were averted between 2001 and 2014 due to malaria interventions LLINs, Indoor Residual Spraying (IRS) and Artemisinin based combination therapy (ACT) (WHO, 2015), with the major share of 68% taken by LLINs followed by ACT (19%) and IRS (13%) (Bhatt et al., 2015). Long-Lasting Insecticidal Nets are designed to provide protection against indoor biting vector mosquitoes. This may lead to sustained residual transmission even after complete LLINs coverage is achieved due to the fact that it does not prevent infectious bites from outdoor biting vector mosquitoes (Killeen, 2014). Thus other complementary tools should be put in place in order to sustain the gains obtained from LLINs/IRS and move towards the envisaged goal of malaria elimination (WHO, 2015). One such tool is repellent application with the objective of addressing people which are not directly protected by LLINs and particularly stay outside in times when vector species are actively foraging (Killeen, 2014).

Repellents can be applied directly to the skin in the form of creams, lotions, oils, powders and aerosols (Fradin and Day, 2002). It can also be prepared in the form of impregnated clothing or on mosquito nets (Bhatnagar and Mehta, 2007). DEET is the most effective, best studied, gold standard synthetic mosquito repellent and it can provide protection up to 6-8 hours if applied properly (Fradin and Day, 2002). It is available in 5% to 100% concentrations in different formulations including solution, lotion, cream, gel, aerosol and pump sprays (Debboun et al., 2007). Essential oils from plants such as citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint have been documented for their repellent activity against mosquitoes (Grainger and Moore, 1991). However, they are less efficacious as their activity is limited to not more than an hour (Grainger and Moore, 1991). The efficacy of repellents can be improved if supplemented with other vector pulling factors such as outdoor attractants to lure and trap/kill mosquitoes, applying insecticides to natural sugar sources and applying topical or systemic insecticides for livestock that kill mosquitoes during or after feeding (WHO, 2014).

The importance of strategic placing of livestock with the purpose of diverting blood seeking potentially infectious mosquitoes away from human (i.e., zooprophylaxis) has been documented since long (Donnelly et al., 2015; WHO, 1982) elsewhere. Complementing non-toxic repellents with an appropriate form of zooprophylaxis, particularly in areas where

zoophilic vector species predominate, may effectively reduce malaria transmission (Killeen et al., 2014b; Poche et al., 2015).

In Ethiopia, a study conducted in the southern part of the country by Habtewold et al. (2001; 2004) showed that using cattle with or without insecticide treatment significantly decreased mosquito biting pressure. Moreover, Massebo et al. (2015) documented the zoophagic behavior of vector species from the same region. However, studies assessing the relationship between applying repellent to human and simultaneously using livestock as pulling factor in order to interrupt potential infectious bites has not been studied. Thus, in this study we took one candidate repellent recently developed at ICIPE Kenya, one commercial plant derived cream formulation repellent from a local pharmacy and DEET to check their efficacy using arm-in-cage experiments. We also studied the push-pull relationship between cattle and human volunteers using repellents in a semi-field setup.

6.3 Materials and Methods

6.3.1 Repellents

Three different repellents, namely Mozigone, Buzz off and DEET were made ready prior to the experiment. Mozigone (5% WLFM-38D, ICIPE, Nairobi, Kenya), a candidate repellent formulation in the form of a cream ointment was obtained from ICIPE, Kenya. Buzz off® (petroleum jelly, essential oil blend, Green PLC, Addis Ababa, Ethiopia), a commercial cream ointment repellent was purchased from a local pharmacy in Jimma, Ethiopia. DEET (Moskito® travel spray, DEET, 30g/100g, 100ml) was obtained from a local supermarket, Addis Ababa, Ethiopia. DEET is a synthetic chemical repellent whereas the other two are plant derived essential oil based repellents.

6.3.2 Study design

6.3.2.1 Experiment I: Adult mosquito rearing and repellency assay

This experiment was conducted in the tropical infectious disease research center, Sekoru campus, Ethiopia in May 2016. Arm-in-cage repellent testing assays were conducted using two different mosquito populations. The first phase of testing was done using a laboratory established reference colony of *An. arabiensis* (WHO, 2009). The second phase of testing was done using a field *An. arabiensis* population raised from field collected larvae. Anopheline larvae were collected from field breeding sites such as small water collects in open fields, pits

dug for house plastering, pits dug for brick making and ditches and brought to the insectary where they were reared into adult under standard conditions (Looker and Taylor-Robinson, 2013). Adult Anopheles mosquitoes were provided with a cotton pad sponged with a 10% sucrose solution until commencement of the experiment. Cotton pads were removed 12 hours prior to the experiment in order to starve mosquitoes. The testing was conducted in a separate bioassay room (approximately 6 m × 5 m × 3 m size) under room temperature. Sixteen mosquito cages were prepared and 150-200 non-blood-fed *An arabiensis* females were added to each cage. The cages were made of an aluminum-frame (40 cm × 40 cm × 40 cm) and window screens (mesh size 256) on all side except the aluminum sheet bottom (WHO, 1996). The readiness of mosquitoes to land and/or probe was assessed by inserting an untreated arm into a cage for 30 seconds or until 10 landings/probing were counted. Then eight human volunteers took one repellent and applied it evenly to the part of the right arm located between elbow and fingertip. Concurrently DEET was applied similarly to the left arm. Volunteers wore gloves and armbands in order to expose the area between wrist and elbow. Two cages (one for left hand and the other for right hand) were assigned to each volunteer. Each volunteer was instructed to insert first the right arm to the right cage and wait for three minute. The hand was withdrawn before the 3-minute completion time if bitten or probed by a mosquito. Then, the DEET treated arm was inserted into the left cage and testing was done in the same way. The volunteers re-inserted their arm after 1 hour, if not bitten in the first round. The testing was continued for 8 hours until occurrence of one landing and/or probing was recorded. At the end of each testing volunteers washed their hands with unflavored soap and dried it with a towel. Each treatment was replicated 48 times using 6 batches of mosquitoes. The entire testing was done using first the laboratory colony of *An. arabiensis* and next repeated with field populations. Complete protection time was calculated as the number of minutes elapsed between the time of repellent application and the first mosquito landing and/or probing. The median complete protection time (CPT) with the 95% confidence interval was estimated from the Kaplan–Meier survival curve (WHO, 2009).

6.3.2.2 Experiment II: Protective efficacy of mosquito repellents in semi-field set up against field populations of Anopheles arabiensis using the release recapture method

Establishment of experimental huts

This experiment was conducted in June 2016 using field experimental huts constructed in the tropical infectious disease research center, Sekoru campus, Ethiopia. Three experimental huts

with size of 5mx5mx 4m were used. Each hut was constructed from brick and cement with the roof made of corrugated iron sheet and ceiling covered with white cotton sheet. Each hut was surrounded by water filled moat in order to prevent the entrance of predator ants in to the system. Two enclosure traps (2m x 1.5m x 2m) made of white sheet and iron frame were erected inside each experimental hut side by side with one-meter space between them following Habtewold et al. (2004). One enclosure trap was assigned to a human volunteer who used repellent and the other to another human volunteer who did not use repellent. A 3x3 Latin square design was used with nights and huts as blocking factors and repellent as treatment.

Adult mosquito rearing and testing procedure

Only anopheline mosquito larvae were dipped and collected from potential breeding sites (Williams and Pinto, 2012). The larvae were reared to adults in Sekoru campus field vector biology laboratory, Sekoru, Ethiopia under standard conditions (temperature 25 ± 2^{o}C, relative humidity 80 ± 4%) (Gerberg et al., 1994; Looker and Taylor-Robinson, 2013). Fifty, 3 to 5 days old, 12 hours starved adult female mosquitoes belonging to *An. gambiae* s.l., presumably *An. arabiensis* (Yewhalaw et al., 2010) were aspirated from a cage and transferred to each of the four labelled cups prior to the testing night. Each cup was labeled by date and hut identification number. At one particular night, 6 volunteers were assigned to 3 huts at 19:00 h and one repellent was randomly assigned to each of the three huts. In each of the three experimental huts one volunteer was assigned to use repellent while the other was not. Volunteers applied repellents first to their palm and then to their hand, face, neck and legs with caution to their eyes and mouth (Fradin, 1998). Between 50 and 55, 3 to 5 days old female mosquitoes were released in the corridor between the two enclosure traps and volunteers enter in to their respective enclosure traps. Then the volunteers close the enclosure traps except for a 30 cm bottom opening/slit for each enclosure trap so that blood meal searching mosquitoes can access the human volunteers through the bottom slits. Each volunteer collected landing/biting mosquitoes from themselves with a flash light and a hand-held mouth aspirator (or mechanical aspirator). Each aspirated mosquito was placed in a pint cup, labeled according to the sampling enclosure trap. Mosquitoes that tended to escape from the house were collected using window exit traps. Mosquitoes were then sorted as alive, dead, unfed and fed. All alive mosquitoes were killed by keeping them at -20 freezers for 10-15 minutes, labeled individually, put in an eppendorf tube (1.5ml), and stored in a plastic box containing desiccant silica gel crystals. Experimental huts were aerated for 24 hours following the collection night.

6.3.2.3 Experiment III: Semi-field trial of additive effect of repellent and zooprophylaxis against field populations of Anopheles arabiensis

This experiment was conducted in June 2016 using field experimental huts constructed in the tropical infectious disease research center, Sekoru campus, Ethiopia and the experimental setup was the same to experiment two except that in this study, one enclosure trap was used to put a calf instead of a human volunteer without repellent. A cattle crush was used to keep the calf in a fixed position while a rectangular pan made of a metal sheet was used to collect urine and droppings. Every night each volunteer involved in data collection entered in the experimental hut at 19:00 h and first closed the door and windows tightly in order to prevent the escape of mosquitoes. Then a calf was tethered in one of the enclosure traps and a 30 cm bottom opening/slit was left open so that blood meal searching mosquitoes could access the hosts. Fifty female mosquitoes were released in the corridor between the two enclosure traps. Human volunteers trained for mosquito collection entered in the remaining enclosure trap and rolled down the sheet except for a 30 cm bottom opening/slit. The human volunteers applied repellent first to their palm and then to their extremities and face with caution to their eyes and mouth (Fradin, 1998). Mosquitoes were collected early in the morning at 06:00 h from the human enclosure trap, the calf enclosure trap, the corridor and exit trap using flash light and mouth aspirator.

6.3.3 Data analysis

The Kaplan–Meier survival curves were used to estimate the median complete protection time for each repellent in experiment one (WHO, 2009). For experiment two and three the data were analyzed by analysis of variance using hut, night and repellent as categorical fixed effects and the difference in number of mosquitoes between the two enclosure traps as response variable. F-tests were applied to test the effect of the repellent. Statistical analysis was done using the SAS software package version 9.3 (SAS Institute Inc., Cary, NC, USA).

6.3.4 Ethical clearance

The objective of the study was explained to the study participants and participants were informed that they had the right to quit at any stage of the study. The participants were briefed that they would be exposed to mosquito bites but that there was no risk of infection due to the fact that all mosquitoes were laboratory raised. Eight volunteers signed a consent form prior to the start of bioassay. All subjects who participated in the study were provided with malaria

prophylaxis as per the national health policy guidelines. This study was cleared by the ethical committee of Jimma University.

6.4 Results

6.4.1 Assessment of complete protection time of candidate repellents vs DEET

The overall complete protection time (CPT) for Buzz off repellent varied between 1 to 62 minutes for both reference and field population of *An. arabiensis*. The median complete protection time (CPT) for Buzz off using both the colony and field population of *An. arabiensis* was 3 minutes. In contrast the DEET repellent, used as positive control, showed a strong repellent activity against both the colony and field populations of *An. arabiensis* with a median CPT of 302 minutes (Table 6.1).

The overall complete protection time for Mozigone varied between 1 minute and 122 minutes for both colony and field populations of *An. arabiensis*. The median CPT for the colony and field populations of *An. arabiensis* was 3 and 61 minutes respectively. In contrast the DEET repellent, used as positive control, showed a strong repellent activity against both colony and field populations of *An. arabiensis.* The median CPT for reference and field population was 303 and 302 minutes respectively (Table 6.2).

Table 6.1 The median complete protection time for Buzz off and DEET using field and lab colony population of *Anopheles arabiensis* from southwestern Ethiopia

Mosquito population	Repellent	Median landing/probing time (in minutes)			P-value
		Estimate ± SE	95% Confidence Interval		
			Lower Bound	Upper Bound	
Field populations of *An. Arabiensis*	Buzz off	3.00 ± 0.20	2.61	3.39	<0.001
	DEET	302.00 ± 0.35	301.32	302.68	
Reference populations of *An. arabiensis*	Buzz off	3.00 ± 0.13	2.74	3.26	<0.001
	DEET	302.00 ± 9.03	284.29	319.70	

Table 6.2 The median complete protection time for Mozigone and DEET using field and lab colony population of *Anopheles arabiensis* from southwestern Ethiopia

Mosquito population	Repellent	Median landing/probing time (in minutes)			P-value
		Estimate ± SE	95% Confidence Interval		
			Lower Bound	Upper Bound	
Field populations of *An. arabiensis*	Mozigone	61.00 ± 0.43	60.15	61.85	<0.001
	DEET	302.00 ± 0.38	301.25	302.740	
Reference populations of *An. arabiensis*	Mozigone	3.00 ± 0.14	2.73	3.27	<0.001
	DEET	303.00 ± 0.35	302.31	303.69	

6.4.2 Protective efficacy of mosquito repellents in semi-field set up against field populations of *Anopheles arabiensis* using release recapture method

An estimated total of 880 mosquitoes were released inside 4 huts in all collection nights. A total of 803 (91%) mosquitoes were retrieved throughout the study period, out of which 213 (26.5%) were fed. Significantly more mosquitoes were collected from the enclosure trap without repellent compared to the enclosure trap with repellent for any of the three repellents. The difference in mosquito densities between the enclosure trap without repellent and the enclosure trap with repellent was equal to 15.25 ($P < 0.001$) for Mozigone, 6.25 ($P = 0.045$) for Buzz off and 9.75 ($P = 0.008$) for DEET. Mean mosquito density per trap or compartment is presented in Table 6.3.

Table 6.3 Mean density of *Anopheles arabiensis* (standard error) in compartment with human with and without repellent in the semi field setup release-recapture method

Repellent	Enclosure trap/compartment			
	Human with repellent	Human without repellent	Corridor	Exit trap
Mozigone	9.75 (2.51)	25.00 (1.40)	14.50 (2.66)	1.75 (0.50)
Buzz off	11.75 (2.51)	18.00 (1.40)	19.50 (2.66)	1.00 (0.50)
DEET	2.25 (2.51)	12.00 (1.40)	33.00 (2.66)	1.75 (0.50)

6.4.3 Protective efficacy of repellents applied to human volunteers when paired with calf used as luring factor

Out of 880 mosquitoes released, 790 (90%) of them were retrieved and 320 (41%) of them were fed. Significantly more mosquitoes were collected from the enclosure trap with a calf (without repellent) compared to the enclosure trap with repellent for DEET and mozigone, but not for buzz off. The difference in mosquito numbers between the enclosure trap with a calf without repellent and the enclosure trap with repellent was equal to 11.75 (P = 0.027) for Mozigone, 8.75 (P = 0.074) for Buzz off and 18.75 (P = 0.004) for DEET. Mean mosquito densities per trap or compartment are given in Table 6.4.

Table 6.4 Mean density of *Anopheles arabiensis* (standard error) in compartment with human with repellent and cattle in the semi field setup release-recapture method

Repellent	Enclosure trap/compartment			
	Human with repellent	Cattle	Corridor	Exit trap
Mozigone	5.75 (1.73)	17.50 (2.94)	19.00 (3.41)	5.75 (1.45)
Buzz off	12.00 (1.73)	20.75 (2.94)	13.75 (3.41)	2.50 (1.45)
DEET	6.75 (1.73)	25.50 (2.94)	18.00 (3.41)	0.25 (1.45)

6.5 Discussion

Control of malaria vector mosquitoes is an important tool in the fight against the disease. Repellents remain one of the key personal protection interventions particularly addressing people which are not directly protected by LLINs and stay outside at times when vector species are actively foraging (Killeen, 2014a). There are mosquito repellents approved by CDC to be applied to skin including synthetic repellents such as DEET, ethyl butylacetylaminopropionate (IR3535®) (Patel et al., 2012) and plant derived oils of Citronella, Lemon and Eucalyptus (Kuehn, 2005). Despite their proven efficacy in personal protection, the usage of synthetic repellents such as DEET is less practiced in vulnerable communities in Africa due to lack of awareness (Govere et al., 2000; Mazigo et al., 2010), affordability (Sangoro et al., 2014) and health related risks (Katz et al., 2014). In this study we evaluated the efficacy of Mozigone, Buzz off and DEET. The evaluation of each repellent was done first using arm-in-cage laboratory experiment and next semi-field setups using experimental huts.

Evaluation of Buzz off using arm-in-cage experiments showed that its protection strength was limited to less than one hour. Fifty percent ended within three minutes. Assessment of the protective efficacy of Buzz off in semi-field set up using two human volunteers (with and without repellent) showed that application of the repellent was associated with a significant decline in biting pressure. Pairing a calf (without repellent) with a human (with repellent) in the semi-field setup resulted also in a reduction of mosquito density in the enclosure traps containing human volunteers, yet the difference was not statistically significant. Plant derived essential oil repellents are short lived in their effect since the essential oils can completely evaporate within a short period (Patel et al., 2012). The synergetic effect of Buzz off with zooprophylaxis has never been reported to the best of our knowledge but combining Buzz off with LLINs was observed to be associated with a reduced risk of malaria infection in the southern part of Ethiopia (Deressa et al., 2014). The usage of water soluble plant based lotion NO MAS (NM) was also associated with significantly lower vector biting burden and reduced prevalence of malaria in Ghana (Dadzie et al., 2013)

Mozigone showed short-lived but relatively better protection as compared to Buzz off with its median complete protection equal to one hour against field populations of *An. arabiensis.* Evaluation of its protective efficacy in semi-field setup using human volunteers (with and without repellent) showed a significant reduction of mosquitoes in the enclosure traps containing human volunteers who did use the repellent. Similarly, significantly lower mosquito density was recorded in enclosure traps that contain human volunteers using Mozigone as

compared to mosquito density from the enclosure traps containing a calf. Due to the observed short lifespan of candidate repellents such as Mozigone, they should be applied repeatedly in order to maximize protection. Research results on a similar use of Mozigone are lacking and can thus not be compared with the current results.

The DEET repellent showed strong repellent activity (3 to 7 hours) against both reference and field populations of *An. arabiensis*. Significantly lower mosquito density was recorded in the enclosure trap containing DEET using human volunteers. Similarly, introduction of a calf significantly reduced the density of mosquitoes recorded in the enclosure traps containing DEET using human volunteers. The protective efficacy of DEET lotion against bites of *An. gambiae* and *An. arabiensis* was well documented in both semi-field setup (82%) and field (93%) from Tanzania when topically applied to human volunteers (Sangoro et al., 2014a) and Pakistan Afghan refugee camp (Rowland et al., 2004). In contrast, the combined treatment of 15% DEET and LLIN did not differ significantly with respect to vector biting from the treatment receiving only LLIN in Tanzania (Sangoro et al., 2014b). Mass distribution of repellents (picaridin) in combination with LLINs made no difference in malaria incidence compared to the control group with LLINs in Cambodia, probably due to no adherence and inappropriate use of the repellents remain the main challenge (Sluydts et al., 2016).

The one-meter space difference between two enclosure traps within an experimental hut should be further optimized in order to minimize the spatial effect of the repellent from treatment enclosure traps.

6.6 Conclusion

Both laboratory and semi-field experiments showed that the protective efficacy of Buzz off (plant based essential oil blend) was documented to be less than one hour which was far below its intended protection time of 8-11 hours. Mozigone (plant based essential oil) provided relatively short but better protection as compared to Buzz off and its bio-prospective aspects should be further examined using field study. Both Buzz off and Mozigone are short lived repellents. In this study calf proved to be a good candidate in diverting away potentially infectious bites. Therefore, it can be used in future studies that involve zooprophylaxis. DEET remains the most effective personal protection repellent amongst the three investigated repellents and should thus be best combined with a zooprophylactic strategy.

Acknowledgements

Jimma University VLIR-IUC program is acknowledged for logistic and financial support. The authors are grateful to Asendebo Vector Biology Laboratory staffs for their excellent technical support.

6.7 References

Bhatnagar, A. and Mehta, V.K. (2007). Efficacy of deltamethrin and cyfluthrin impregnated cloth over uniform against mosquito bites. *Med J Armed Forces India* 63, 120-122.

Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Brie¨t, O., Penny, M.A., Smith, T.A., Bennett, A., Yukich, J., Eisele T.P., Griffin J.T., Fergus C.A., Lynch M., Lindgren F., Cohen J.M., Murray C.L.J., Smith D.L., Hay S.I., Cibulskis R.E. and Gething P.W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207.

Dadzie, S., Boakye, D., Asoala, V., Koram, K., Kiszewski, A. and Appawu, M. (2013). A community wide study of malaria reduction: evaluating efficacy and user acceptance of a low-cost repellent in northern Ghana. *Am. J. Trop. Med. Hyg.* 88, 309-314.

Debboun, M., Frances, S.P. and Strickman, D. (2007). Insect repellents: principles, methods, and uses. CRC, Boca Raton, FL.

Deressa, W., Yihdego, Y.Y., Kebede, Z., Batisso, E., Tekalegne, A. and Dagne, G.A. (2014). Effect of combining mosquito repellent and insecticide treated net on malaria prevalence in Southern Ethiopia: a cluster-randomized trial. *Parasites Vectors* 7, 132.

Donnelly, B., Berrang-Ford, L., Ross, A.N. and Michel, P. (2015). A systematic, realist review of zooprophylaxis for malaria control. *Malar. J.* 14, 313.

Fradin, M.S. (1998). Mosquitoes and mosquito repellents: A clinician's guide. *Ann. Intern. Med.* 128, 931-940.

Fradin, M.S. and Day, J.F. (2002). Comparative efficacy of insect repellents against mosquito bites. *N. Engl. J. Med.* 347, 13-18.

Gerberg, E.J., Barnard, D.R. and Ward, R.A. (1994). *Manual for mosquito rearing and experimental techniques*. American mosquito control association, Inc.

Govere, J.M., Durrheim, D.N., La Grange, J.J.P., Mabuza, A. and Booman, M. (2000). Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga province south Africa. *S. Afr. Med. J.* 90, 611-616.

Grainger, J. and Moore C. (1991). Natural insect repellents for pets, people and plants. Austin: the herb bar.

Habtewold, T., Prior, A.T., Torr, J.S. and Gibson, G. (2004). Could insecticide-treated cattle reduce Afrotropical malaria transmission? Effects of deltamethrin-treated zebu on *Anopheles arabiensis* behavior and survival in Ethiopia. *Med. Vet. Entomol.*18, 408-417.

Katz, T.M., Miller, J.H. and Hebert, A.A. (2014). Insect repellents: historical perspectives and new developments. *J. AM. Acad. Dermatol.* 58, 865-71.

Killeen, F.G. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar. J.* 13, 330.

Killeen, G.F., Seyoum, A., Gimnig, J.E., Stevenson, J.C., Drakeley, C.J. and Chitnis, N. (2014). Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. *Malar. J.* 13, 146.

Kuehn, B.M. (2005). CDC: New repellents for west Nile fight. *JAMA* 293, 2583.

Looker, M. and Taylor-Robinson A.W. (2013). Mosquitoes and parasites. In: Kirsten Moll, Akira Kaneko, Arthur Scherf and Mats Wahlgren (Eds). *Methods in malaria research*. 6th Ed. Manassas, VA, USA: MR4/ATCC

Mazigo, H.D., Obasy, E., Mauka, W., Manyiri, P., Zinga, M., Kweka, E.J., Mnyone, L.L. and Heukelbach J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. *Malar. Res. Treat.* 2010, 794261

Patel, E.K., Gupta, A. and Oswal, R.J. (2012). A review on: mosquito repellent methods. *Int. J. Pharm. Chem. Biol. Sci.* 2, 310-317.

Poche, R.M., Burruss, D., Polyakova, L., Poché, D.M. and Garlapati, R.B. (2015). Treatment of livestock with systemic insecticides for control of *Anopheles arabiensis* in western Kenya. *Malar. J.* 14, 351.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J., Fayaz, M. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop. Med. Int. Health* 9, 335-342.

Sangoro, O., Lweitojera, D., Simfukwe, E., Ngonyani, H., Mbeyela, E., Lugiko, D., Kihonda, J. and Moore S. (2014a). Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data. *Malar. J.* 13, 159.

Sangoro, O., Turner, E., Simfukwe, E., Miller, J.E., Moore, S.J. (2014b). A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission. *Malar. J.* 13, 324.

Sluydts, V., Durnez, L., Heng, S., Gryseels, C., Canier, L., Kim, S., Van Roey, K., Kerkhof, K., Khim, N., Mao, S., Uk, S., Sovannaroth, S., Grietens, K.P., Sochantha, T., Menard, D. and Coosemans M. (2016). Efficacy of topical mosquito repellent (picaridin) plus long-lasting

insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomized controlled trial. *Lancet Infect. Dis.* 16, 1169-1177.

WHO. (1982). *Manual on environmental management for mosquito control with special emphasis on malaria vectors.* World health organization. Geneva, Switzerland

WHO. (1996). *Report of the WHO informal consultation on the evaluation and testing of insecticides.* [online] available from: CTD WHOPES_IC_96.1.pdf [accessed: 07/25/2016]

WHO. (2009). *Guidelines for efficacy testing of mosquito repellents for human skin*. World health organization. Geneva, Switzerland.

WHO. (2014). *Control of residual malaria parasite transmission: guidance note.* [online] available from WHO/HTM/GMP/MPAC/2014.5 [accessed: 07/25/2016]

WHO. (2015). World malaria report. World health organization, Geneva. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed: 04/04/2016].

Williams, J. and Pinto, J. (2012). Training manual on malaria entomology: for entomology and vector control technicians (Basic Level). United States agency for international development. [Online].Availablefrom:http://www.rollbackmalaria.org/files/files/partnership/wg/wg_itn/docs/ws8/TrainingManualOnMalariaEntomology-en.pdf [accessed on: 2/27/16]

Yewhalaw, D., Bortel, V.W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Am. J. Trop. Med. Hyg.* 83, 122-125.

Chapter 7

General discussion

7.1 Overview

The assessment of the impact of insecticide resistance on malaria vector control interventions (LLINs and IRS) in Ethiopia using DDT and PermaNet 2.0 in field experimental huts showed that there was no significant difference in mosquito blood feeding rates between sprayed and unsprayed hut. There was also no significant difference in mosquito blood feeding rates between a hut with treated net and a hut with untreated net. Moreover, the mean exit rate was similar for sprayed and unsprayed hut and between a hut with treated net and a hut with untreated net. There was no difference in mosquito mortality between sprayed and unsprayed hut nor between a hut with LLIN and a hut with untreated net.

The assessment of the host preference of *An. arabiensis* using three alternative experimental setups in southwestern Ethiopia showed that populations of *An. arabiensis* from Jimma area were zoophilic. The density of *An. arabiensis* was significantly higher in the enclosure trap with calf as compared to the enclosure trap with human in all three experimental setups. In contrast in all three experimental setups the density of *An. arabiensis* was significantly lower in the enclosure trap with chicken as compared to the enclosure trap with human.

The additive value of repellents (Buzz off, Mozigone and DEET) as pushing factor and cattle as a pulling factor was assessed using the release-recapture method in a controlled semi-field system. Significantly higher mosquito density was recorded in enclosure traps without repellent as compared to enclosure traps containing human volunteers applying Mozigone and DEET as compared to mosquito density recorded in the enclosure traps of human without repellent and enclosure traps containing calf.

Thus, it appears that the emergence of insecticide resistance, along with the lack of diversified intervention tools, jeopardizes the renewed call for malaria elimination. In this general discussion we describe how the fight against malaria has paid off at first but that the envisaged elimination is challenged now due to insecticide resistance and changing behavior of the mosquito population. We further describe improved intervention tools that could be helpful in coping with the changing status and behavior of the malaria vector population.

7.2 The global malaria elimination agenda

In the first half of the 20th century, malaria was endemic in most countries and territories (148) of the world, affecting about 90% of the world's population and reaching as far north as the Arctic Circle (Feachem et al., 2010; Karunamoorthi, 2011). Supported by successful efforts to

reduce malaria with dichlorodiphenyltrichloroethane (DDT), the World Health Organization launched the Global Malaria Eradication Campaign (1955-1969) in the 8th World Health Assembly held in 1955 for all malarious countries based primarily on interventions with DDT as a vector control tool together with case management (WHO, 2008; Karunamoorthi, 2011). That program was suspended due to the emergence of drug resistance, lack of diversified intervention tools and the contraction of funding and lack of political commitment in the 1970's and was replaced by the WHO by a new program to control the disease (Najera et al., 2011, WHO, 1969). Since then, the global malaria incidence came down substantially despite exponential population growth in malaria-endemic areas during the past 60 years. Today an estimated 50% of the world's population lives in malaria-free areas, compared with only 30% in 1950 (Hay et al., 2004; Guerra et al., 2008; Feachem et al., 2010).

Seventy-nine countries have eliminated malaria between 1945 and 2016. Thirty-eight of them have been certified and declared as malaria free in the WHO official register as having eliminated malaria through specific measures (Feachem et al., 2010; WHO, 2016). Today there are 106 countries with ongoing malaria transmission (WHO, 2015a) of which 32-35 are pursuing elimination and the remaining countries are controlling (Das and Horton, 2010). The dramatic decline in both disease morbidity and mortality is accompanied by a shrinking global malaria incidence map as the disease is now mostly confined to the tropical world (Feachem et al., 2010, WHO, 2016).

The scaling up of malaria control efforts, including LLINs, IRS, ACT and rapid diagnostic tests (RDT) together with an increase in finances for malaria has resulted in progress towards elimination in several countries since the early part of the 21st century and inspired the World Health Organization to envisage to eliminate malaria (Feachem et al., 2010). Malaria elimination is defined as a state where interventions have interrupted endemic transmission and limited onward transmission from imported infections below a threshold at which risk of re-establishment is minimized. Both capacity and commitment to sustain this state are required indefinitely (Cohen et al., 2010). Similarly, the World Health Organization sets criteria to member states to pass through all four pathways including control, pre-elimination, elimination and prevention of re-establishment to be certified as malaria free state or territory (WHO, 2016).

As part of a step towards elimination, the World Health Assembly adopted in 2015 the Global Technical Strategy for Malaria 2016-2030 (GTS), a 15-year strategic action plan for malaria control and elimination. The strategic plan was developed by the "Roll Back Malaria" program in partnership with the advocacy plan "Action and Investment to Defeat Malaria 2016- 2030"

(AIM). As part of the plan global malaria cases will be reduced by 40% by 2020, 75% by 2025 and 90% by 2030 respectively. In line with this, 10 countries are expected to be to certified as malaria free by 2020, another 10 by 2025 and a total of 35 states by 2030 (WHO, 2016; Newby et al., 2016). The number of states that could work towards elimination according to the current performance is expected to be around 32-34 (Feachem et al., 2010; Cotter et al., 2013).

But the fight is far from over and faces a lot of roadblocks. New threats in malaria vector control have however recently appeared apart from the emergence of insecticide resistance (Killeen et al., 2014)., there are some hard to reach populations including ethnic or political minority groups, which are typically impoverished and not mobile, often driven to more remote areas by marginalization and safety concerns (Martens and Hall, 2000). Delivery of services to this group of people can be challenging because their identities vary by setting and their members often face substantial barriers to health-care access (Hiwat et al., 2012; Chuquiyauri et al., 2011). One of the many challenges facing malaria eliminating countries is, the re-establishment of malaria due to imported malaria cases from neighboring high-endemic areas (Abeyasinghe et al., 2012). In addition to imported malaria cases, the *Plasmodium vivax* parasite can survive in a dormant liver stage, which can result in relapses even a long time after the last *Plasmodium vivax* clinical malaria case (Meuller et al., 2009).

7.3 Vector control remains a corner stone in malaria control

Vector control activities involve mainly three interventions, namely personal protection interventions, IRS and environmental management (Karunamoorthi, 2011). Long-lasting insecticidal nets and repellent formulations or repellent clothing are mostly available personal protection interventions. Indoor residual spraying (IRS) remains one of the oldest vector control methods used in malaria vector control and this includes spraying indoor spaces with selected organochlorines, organophosphates, carbamates and pyrethroids (WHO, 2015b). Environmental management, on the other hand, includes draining potential larval breeding habitats, spraying breeding sources, changing housing setup (screening windows and doors), strategic placement of livestock and other environmental management strategies (WHO, 2013). The concerted efforts in the development and introduction of protective bed nets in the late 1990's and its mass distribution since the beginning of 2000 has contributed to the aversion of millions of malaria cases and deaths (Bhatt et al., 2015; WHO, 2015a). The LLINs are impregnated with pyrethroid chemicals (WHO, 2004) that are supposed to kill vector mosquitoes upon contact and are recently further strengthened with coating of synergist

chemicals such as piperonyl butoxide (PBO) in order to target pyrethroid resistant vector populations (CDC, 2010). Synergist chemicals are not insecticides by themselves but they inhibit enzymes responsible types of resistance (CDC, 2010; Tungu et al., 2010). While the distribution of LLINs and coverage of IRS should be widely continued in the control effort of the vector population, there should be regular entomological monitoring and testing of the bio efficacy of the products in order to verify whether they are performing up to the set standard.

In Ethiopia, vector populations have developed resistance to three classes of insecticides including organochlorines, organophosphates and phyrethroids (Yewhalaw et al., 2010; 2011, Balkew et al., 2010). Moreover, bottle bioassay tests on pyrethroids (permethrin and deltamethrin) and WHO cone bioassay tests conducted on net sections taken from LLIN (PermaNet 2.0) confirmed that vector populations have reduced susceptibility (Yewhalaw et al., 2012). However, no field studies have been conducted in order to evaluate whether the reported insecticide resistance has implications on the current vector control interventions (ITN and IRS) in the country. Therefore, we evaluated the efficacy of both IRS (DDT) and LLINs (PermaNet 2.0) using field experimental huts (WHO, 2006). In our experiment we deliberately made holes in the nets (both LLINs and untreated nets) to assess whether the coated insecticide not the net itself is effectively preventing mosquito bites. Our results showed that mosquitoes are not responding to the pyrethroid chemicals coated on the surface of the net as no significant difference was observed in the number of mosquitoes fed on human volunteers protected by LLINS inside experimental huts as compared to the number of mosquitoes fed on human volunteers protected by untreated nets inside experimental huts. Similarly, there was no significant difference in the number of fed mosquitoes collected from DDT sprayed huts as compared to the number of fed mosquitoes collected from unsprayed hut (Chapter 4). DDT was banned from public health utilization in the 1970's. However, its application was recommended again by WHO to African states considering the disease burden in the continent (WHO, 2006; Weissman, 2006). Ethiopia has been using DDT for many years and discontinued its application in 2009 (Bisco et al., 2004). Initially it was replaced by deltamethrin and following resistance reports deltamethrin was replaced by bendiocarb. Currently the application of bendiocarb is mostly limited because of the emergence of resistance; instead pirimiphos methyl, (organophosphate) and propoxur (carbamate) are introduced for IRS by the National Malaria Control program (NMCP) (PMI, 2016). Reduced efficacy results were observed for PermaNet 2.0, which means that the national malaria control programs need to re-assess its efficacy and that PermaNet 2.0 probably needs to be replaced with better performing LLINs such as PermaNet 3.0 (Tungu et al., 2010). Long-lasting insecticidal nets

(LLINs) were developed as a more sustainable solution to the limitations encountered with conventional treated nets (CTNs) (removing the need for regular re-treatment of impregnated nets with insecticide) and are expected to retain biological activity for at least 20 standard WHO washes under laboratory conditions and three years of use under field conditions. The evolution of pyrethroid resistance in the vector population could limit the efficacy of LLINs (N'Guessan et al. 2007; Ngufor et al., 2011; Corbel et al., 2010). Whether the reported *kdr* is the only responsible mechanism for the observed reduced efficacy of LLINs in the current study or whether it is due to the combination of other resistance mechanisms needs to be further investigated.

Another drawback of LLINs and IRS is that they are designed to provide protection against indoor biting and indoor resting vector mosquitoes. This may lead to sustained residual transmission even after full LLINs coverage and IRS is achieved due to its limitation to address outdoor biting vector mosquitoes (Killeen et al., 2014). Moreover, it is now well established that some vector species (for instance *An. arabiensis*) show behavioral plasticity in terms of host preference, resting places and biting pattern (Maxwell et al., 1998; Shililu et al., 2004; Killeen et al., 2006) and by doing so these vectors can easily escape/evade contact with insecticide treated surfaces to maintain a certain level of transmission (Durnez and Coosemans, 2013).

In Ethiopia the dominant vector species is *An. arabiensis*, a member of the gambiae complex. Studies have proven that *An. arabiensis* has varying behavior (biting time, blood meal source and resting place) depending on the circumstances. It is an opportunistic feeder with a broad host range (Massebo et al., 2015; Duchemin et al., 2001; Hadis et al., 1997; Waka et al., 2005; Tirados et al., 2006; Fornadel et al., 2010; Fontenille et al., 1997), a varying biting pattern (Yohannes and Boelee, 2012) and is resting both indoor and outdoor (Taye et al., 2016). Due to this behavioral plasticity it is difficult to control this vector by the currently available control methods IRS and LLINs. Therefore, we evaluated the potential of zooprophylaxis in reducing the human-vector contact as a complementary intervention in Ethiopia (Chapter 5). To that end, the mosquito density differences attracted between enclosure traps with human and livestock hosts (calf, goats, donkeys, and chicken) were assessed in semi-field set ups and used as a measure of preference.

The results from the three different experimental setups showed that populations of *An. arabiensis* from Jimma area preferred to feed on cattle as compared to human. The outcome is less apparent for the other two livestock hosts (equine and ovine). Thus, cattle could have a role in diverting malaria vectors away from human and thus reduce the human-vector contact

in vector control interventions (Chapter 5). The vectorial capacity of anophelines mainly depends on their preference to feed on humans and their susceptibility to *Plasmodium* (Lefevre et al., 2009). Host preference can be inherent or induced (Killen et al., 2014). There are many environmental factors, acting in combination with the innate preference, that determine the final host selection. This may include host availability, host accessibility, and the previous feeding experience of the mosquito (Killen et al., 2001; Lefevre et al., 2009).

Understanding the host preference behavior of a vector species is key in developing novel vector control tools such as attractants which may be deployed in mass trapping (Besansky et al., 2004) and luring the vectors to a certain point as is the case in zooprophylaxis. In addition to luring and killing vector mosquitoes it is also possible to couple attractants and repellents.

In our quest for alternative vector control intervention tools, we further investigated the additive effect of repellents and zooprophylaxis. Cattle can be potentially used for zooprophylaxis in this particular region as we demonstrated in our first experiment (i.e., *An. arabiensis* is more attracted to calf than to human and further confirmed with analysis of blood meal source). One of the key problems in residual transmission is that vectors feed indoor and rapidly exit the housing structures avoiding resting on sprayed surface before picking lethal doses (Reddy et al., 2011). Mosquitoes also bite people when they are engaged in both indoor and outdoor activities in early evening such as irrigation farming, cattle keeping, avoiding excess heat, and staying in recreational centers (Pates and Curtis, 2005). Thus, the time period between 18:00 h and 22:00 h has been reported the most critical in residual transmission (Chaccour and Killeen, 2016).

Hence, we assessed the efficacy of different available repellents (Chapter 6). Our studies revealed that plant derived essential oil blend Mozigone, prepared in the form of a cream, provided relatively better protection up to 120 minutes as compared to Buzz off which provided protection for less than one hour. DEET remains the only reliable personal protection currently available in the country. It provides protection by repelling mosquitoes for longer time periods up to 7 hours. Here we also report significantly lower density of mosquitoes collected from enclosure traps of human volunteers applying Mozigone and DEET repellent as compared to the density of mosquitoes collected from enclosure traps with calf. Entomological studies conducted on the effect of topical repellents against malaria vector biting activities showed that repellents can provide significant reduction in biting pressure (Govere et al., 2000; Dadzie et al., 2013). However, the observed protection against vector biting at individual level is not directly transferrable to reduction in parasitaemia at the community level as demonstrated in large-scale trials (Chen-Hussey et al., 2013; Sangoro et al., 2014, Sluydts et al., 2016; Wilson

et al., 2014). Studies were conducted at different levels of randomization (individual, household, community wide cluster) and have failed to show significant reduction in risk of infection except for a household randomized trial from Pakistan (Rowland et al., 2004) using DEET and a community based cluster randomized trial from Ethiopia (Deressa et al., 2014) with a combined intervention of Buzz off and LLINs. Studies were either comparing subjects with and without repellent (McGready et al., 2001; Rowland et al., 2004; Chen-Hussey et al., 2013) or repellent with ITN versus ITN alone (Hill et al., 2007; Sangoro et al., 2014; Deressa et al. 2014; Sluydts et al., 2016). Mostly DEET was used as repellent but also other repellents such as Picaridin and other plant derived repellents have been used. However, the additive effect of repellents and zooprophylaxis has not been studied in the field.

7.4 Zooprophylaxis and repellent in malaria vector control

The emergence and resurgence of vector populations that defy the main control methods LLINs and IRS makes the prospect of malaria elimination doubtful (Durnez and Coosemans, 2013; Killeen et al., 2014). The lack of diversified vector control tools, particularly to target outdoor biting vector species, is the driving factor behind the search for innovative vector control tools. Zooprophylaxis is an old approach but it can be modified or supplemented with other vector control tools as part of integrated vector management.

Thus, in our study on exploring the potential of zooprophylaxis (Chapter 3 systematic review) we have shown that livestock placed separately at an optimum distance, combined with other interventions such as LLINs (Kaburi et al., 2009; Iwashita et al., 2014; Killeen and Smith, 2007), livestock treated with insecticide, (Lyimo et al., 2012; Mahande et al., 2007; Rowland et al., 2001; Hewitt and Roland, 1999), cattle treated with ivermectin (Fritz et al., 2009; Foley et al., 2009), can readily reduce the risk of malaria infection. Furthermore, in our current study we have shown that in the presence of zoophilic vectors such as *An. arabiensis*, livestock could significantly reduce potentially infectious bites from human, especially when treated with good repellents such as DEET.

Repellents with short life span up to 2 to 3 hours, if used properly, can provide protection from early evening infectious mosquito bites (18:00 h - 22:00 h) which is the time where people remain outdoor (Yohannes and Boelee, 2012). We have shown that DEET and Mozigone could be an alternative to tackle residual transmission (Killeen, 2014) if combined with LLIN (Hill et al., 2007) and zooprophylaxis. Compliance (Sluydts et al., 2016), awareness (Govere et al.,

2000; Mazigo et al., 2010), affordability (Sangoro et al., 2014) and health related risks (Katz et al., 2014) are the major challenges in applying repellents in a successful way.

7.5 Future perspectives on vector control tools

In our effort to assess the efficacy of major malaria vector control methods we have shown that the main malaria vector interventions IRS (based on DDT spraying) and LLINs (PermaNet 2.0) have reduced efficacy in Ethiopia. At present, bendiocarb and propoxur are being used for IRS interventions and PermaNet 2.0 and PermaNet 3.0 are being used as LLINs in Ethiopia (PMI-Ethiopia, 2016). While the combination of the two interventions remains critical to achieve the WHO objective to entirely eliminate malaria, it should be investigated whether these vector control interventions alone are sufficient to reduce the malaria incidence to a level where it dies out. Our study was limited to a field experimental huts trial which could not account for some of the factors that could further reduce the efficacy of these interventions. For instance, the efficacy of both LLINs and IRS in actual usage within the community are subjected to weathering, tearing, rubbing smoking and lack of compliance by users. Thus, community wide field randomized control trials should be conducted in order to evaluate whether and to what extent the reported insecticide resistance compromises the current malaria vector control interventions.

The host preference of populations *An. arabiensis* from southwestern Ethiopia is zoophilic with the mosquitoes mainly preferring to feed on cattle (Asale et al., 2016), equally exophagic and endophagic (Taye et al., 2016). Thus, zooprophylaxis could be a promising supplementary vector control intervention to be used in the area. To that end, factors such as the distance between human quarters and animal shed (structure) and door-window screening technologies need to be optimized. Furthermore, other factors such as the distance to larval breeding site should be considered. Combining the proposed strategy with other interventions, such as the treatment of livestock with non-repellent insecticides and endectocide treated cattle, can further strengthen this approach and thus needs to be further investigated. Our current study on host preference is mainly focused on measuring entomological parameters using the semi-field setup. However, the endpoint of zooprophylaxis, i.e. controlling malaria using livestock as a protective barrier should be further tested at the community level by measuring the association between malaria incidence and the possession of livestock. There is growing evidence that host preference of a mosquito is influenced by its previous blood meal source (Takken and Verhulst, 2012) and whether it is infected by the *Plasmodium* parasite (Cator et al., 2012). Thus, the

effect of previous blood meal and *Plasmodium* infection on the next host choice of vector mosquitoes should be further investigated using the biosphere semi-field set up.

Vector biting that could result in infections in the early evening hours (18:00 h-22:00 h) is less or not tackled by the main vector control interventions IRS and LLINs as people especially in Africa quite often remain outside being engaged in several activities (Yohannes and Bolee, 2012). Repellents such as Mozigone and DEET can be used to protect humans from the early evening mosquito bites. Combining repellents with zooprophylaxis showed good prospects and could become an important component in integrated vector management. However, larger scale randomized controlled field trial should be carried out on these tools with and without combination as current evaluations are limited to laboratory and semi-field set ups.

7.6 References

Abeyasinghe, R.R., Galappaththy, G.N.L., Smith, G.C., Kahn, J.G., Feachem, R.G.A. (2012). Malaria control and elimination in Sri Lanka: documenting progress and success factors in a conflict setting. *PLoS One* 7, e43162.

Animut, A., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2013). Blood meal sources and entomological inoculation rates of anophelines along a highland altitudinal transect in south-central Ethiopia. *Malar. J.* 12, 76.

Asale, A., Getachew, Y., Hailesilassie, W., Speybroeck, N., Duchateau, L. and Yewhalaw D. (2014). Evaluation of the efficacy of DDT indoor residual spraying and long-lasting insecticidal nets against insecticide resistant populations of *An. arabiensis* Patton (Diptera: Culicidae) from Ethiopia using experimental huts. *Parasites Vectors* 7, 131.

Balkew, M., Ibrahim, M., Koekemoer, L.L., Brooke, B.D., Engers, H., Aseffa, A., Gebre Michael, T. and El-Hassen, I. (2010). Insecticide resistance in *An. arabiensis* (Diptera: Culicidae) from villages in central, northern and southwest Ethiopia and detection of kdr mutation. *Parasites Vectors* 3, 40.

Besansky, N.J., Hill, C.A. and Costantini, C. (2004). No accounting for taste: host preference in malaria vectors. *TRENDS in Parasitology* 20, 249-252.

Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Brie¨t, O., Penny, M.A., Smith, T.A., Bennett, A., Yukich, J., Eisele, T.P., Griffin, J.T., Fergus, C.A., Lynch, M., Lindgren, F., Cohen, J.M., Murray, C.L.J., Smith, D.L., Hay, S.I., Cibulskis, R.E. and Gething, P.W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526, 207.

Bouma, M. and Rowland M. (1995). Failure of passive zooprophylaxis: cattle ownership in Pakistan is associated with a higher prevalence of malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 89, 351-353.

Cator, L.J., Lynch, P.A., Read, A.F. and Thomas, M.B. (2012). Do malaria parasites manipulate mosquitoes? *Trends Parasitol.* 28, 466–470.

CDC. (2010). Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. Available from:

Chaccour, C. and Killeen, G.F. (2016). Mind the gap: residual malaria transmission, veterinary endectocides and livestock as targets for malaria vector control. *Malar. J.* 15, 24.

Chen-Hussey, V., Carneiro, I., Keomanila, H., Gray, R., Bannavong, S., Phanalasy, S. and Lindsay, S.W. (2013). Can Topical Insect Repellents Reduce Malaria? A Cluster-Randomised Controlled Trial of the Insect Repellent *N,N*-diethyl-*m*-toluamide (DEET) in Lao PDR. *PLoS ONE* 8, e70664. doi:10.1371/journal.pone.0070664

Chuquiyauri, R., Paredes, M., Penataro, P., Torres, S., Marin, S., Tenorio, A., Brouwer, K.C., Abeles, S., Llanos-Cuentas, A., Gilmand, R.H., Kosek, M. and Vinetz, J.M. (2012). Socio-demographics and the development of malaria elimination strategies in the low transmission setting. *Acta Trop.* 121, 292-302.

Cohen, J.M., Moonen, B., Snow, R.W. and Smith, D.L. (2010). How absolute is zero? An evaluation of historical and current definitions of malaria elimination. *Malar. J.* 9, 213.

Coluzzi, M., Sabatini, A., Petrarca, V. and Di Deco, M.A. (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. R. Soc. Trop. Med. Hyg.* 73, 483-497. doi:10.1016/0035-9203(79)90036-1

Corbel, V., Chabi, J., Dabiré, K.R., Etang, J., Nwane, P., Pigeon, O., Akogbeto, M. and Hougard, J. (2010). Field efficacy of a new mosaic long-lasting mosquito net (PermaNet® 3.0) against pyrethroid resistant malaria vectors: a multi-center study in western and central Africa. *Malar. J.* 9, 113.

Costantini, C., Sagnon, N., Torre, A.D., Della, A. and Coluzzi, M. (1999). Mosquito Behavioral Aspects of Vector-Human Interactions in the *Anopheles gambiae* complex. *Parassitologia* 41,209-17·

Cotter, C., Sturrock, H.J.W., Hsiang, M.S., Liu, J., Phillips, A.., Hwang, J., Gueye, C.S., Fullman, N., Gosling, R.D. and Feachem, R.G.A., (2013). The changing epidemiology of malaria elimination: new strategies for new challenges. *Lancet* 382, 900-911.

Dadzie, S., Boakye, D., Asoala, V., Koram, K., Kisweski, A. and Appawu, M. (2013). A community-wide study of malaria reduction: Evaluating efficacy and user-acceptance of a low-cost repellent in Northern Ghana. *Am. J. Trop. Med. Hyg.* 88, 309-14.

Das, P. and Horton, R. (2010). Malaria elimination: worthy, challenging, and just possible. *Lancet* 376, 1515-1517.

Deressa, W., Ali, A. and Berhane, Y. (2007). Household and socioeconomic factors associated with childhood febrile illnesses and treatment seeking behavior in an area of epidemic malaria in rural Ethiopia. *Trans. Roy. Soc. Trop. Med. Hyg.*101, 939-947.

Deressa, W., Yihdego, Y.Y., Kebede, Z., Batisso, E., Tekalegne, A. and Dagne, G.A. (2014). Effect of combining mosquito repellent and insecticide treated net on malaria prevalence in Southern Ethiopia: a cluster-randomized trial. *Parasites Vectors* 7, 132.

Duchemin, B., Pock Tsy, L-M.J., Rabarison, P., Roux, J., Coluzzi, M. and Costantini, C. (2001). Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odor-baited entry traps. *Med. Vet. Entomol.*15, 50-57.

Durnez, L. and Coosemans, M. (2013). Residual transmission of malaria: an old issue for new approaches. In: *Anopheles mosquitoes - New insights into malaria vectors*. Manguin S., (Ed.) Rijeka, Croatia-EU: INTECH DOO.

Feachem, R.G.A., Phillips, A.A., Hwang J., Cotter C., Wielgosz B., Greenwood B.M., Sabot O., Rodriguez M.H., Abeyasinghe R.R., Ghebreyesus, T.A. and Snow, R.W. (2010). Shrinking the malaria map: progress and prospects. *Lancet* 376, 1566-1578.

Foley, D.H., Bryan, J.H. and Lawrence, G.W. (2009). The potential of ivermectin to control the malaria vector *Anopheles farauti*. *Trans. Roy. Soc. Trop. Med. Hyg.* 94, 625-628.

Fontenille, D., Lochouarn, L., Diatta, M., Sokhna, C., Dia I., Diagne N., Lemasson J.J., Ba K., Tall A., Rogier, C. and Trape, J.F. (1997). Four years entomological study of the transmission of seasonal malaria in Senegal and the bionomics of *Anopheles gambiae* and *An. arabiensis*. *Trans. Roy. Soc. Trop. Med. Hyg.* 91, 647-652.

Fornadel, C.M., Norris, L.C., Glass, G.E. and Norri, D.E. (2010). Analysis of *Anopheles arabiensis* blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. *Am. J. Trop. Med. Hyg.* 83, 848-853.

Fritz, M.L., Siegert, P.Y., Walker, E.D., Bayoh, H.M.N., Vulule, J.R. and Miller, J.R. (2009). Toxicity of blood meals from ivermectin-treated cattle to *Anopheles gambiae s.l. Ann. Trop. Med. Parasitol.* 103, 539-547.

Githinji, S., Herbst, S. and Kistemann, T. (2009). The human ecology of malaria in a highland region of southwest Kenya. *Schattauer* 48, 451-453.

Govere, J., Durrheim, D.N., Baker, L., Hunt, R. and Coetzee, M. (2000). Efficacy of three insect repellents against the malaria vector *Anopheles arabiensis*. *Med. Vet. Entomol.* 14, 441-444. 10.1046/j.1365-2915.2000.00261.x.

Govere, J.M., Durrheim, D.N., La Grange, J.J.P., Mabuza, A. and Booman, M. (2000). Community knowledge and perceptions about malaria and practices influencing malaria control in Mpumalanga province South Africa. *SAMJ S. Afr. Med. J.* 90, 611-616.

Greenwood, B.M. (2008). Control to elimination: implications for malaria research. *Trends Parasitol.* 24, 249-254.

Guerra, C.A., Gikandi, P.W., Tatem, A.J., Noor, A.M., Smith, D.L., Hay, S.I. and Snow, R.W. (2008). The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PLos Med.* 5, e38.

Habtewold, T., Walker, A.R., Curtis, C.F., Osir, E.O. and Thapa, N. (2001). The feeding behaviour and *Plasmodium* infection of *Anopheles* mosquitoes in southern Ethiopia in relation to use of insecticide-treated livestock for malaria control. *Trans. Roy. Soc. Trop. Med. Hyg.* 95, 584-586.

Hadis, M., Lulu, M., Makonnen, Y. and Asfaw, T. (1997). Host choice by indoor-resting *Anopheles arabiensis* in Ethiopia. *Trans. Roy. Soc. Trop. Med. Hyg.* 91, 376-378.

Hay, S.I., Guerra, C.A., Tatem, A.J., Noor, A.M. and Snow, R.W. (2004). The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect. Dis.* 4, 327-36.

Hewitt, S. and Rowland, M. (1999). Control of zoophilic malaria vectors by applying pyrethroid insecticides to cattle. *Trop. Med. Int. Health.* 4, 481-486.

Hill, N., Lenglet, A., Arnéz, A.M. and Carneiro, I. (2007). Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomized placebo controlled clinical trial in the Bolivian Amazon. *Brit. Med. J.* 335, 1023.

Hill, N., Lenglet, A., Arnéz, A.M. and Carneiro, I. (2007). Plant based insect repellent and insecticide treated bed nets to protect against malaria in areas of early evening biting vectors: double blind randomized placebo controlled clinical trial in the Bolivian Amazon. *BMJ* 335, 1023.

Hiwat, H., Hardjopawiro, L.S., Takken, W. and Villegas, L. (2012). Novel strategies lead to pre-elimination of malaria in previously high-risk areas in Suriname, South America. *Malar. J.* 11, 10.

http://who.int/malaria/publications/atoz/cochrane_reviewitns2004.pdf [accessed on: 09/16/2016].

https://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf (accessed on: 09/16/2016).

Idrees, M. and Jan, A.H. (2001). Failure of zooprophylaxis: cattle ownership increase rather than reduce the prevalence of malaria in district Dir, NWFP of Pakistan. *J. Med. Sci.* 1, 52-54.

Iwashita, H., Dida, O.G., Sonye, O.G., Sunahara, T., Futami, K., Njenga, M.S., Chaves, F.L. and Minakawa, N. (2014). Push by a net, pull by a cow: can zooprophylaxis enhance the impact of insecticide treated bed nets on malaria control? *Parasites Vectors* 7, 52.

Jaleta, T.K., Hill, R.S., Birgersson, G., Tekie, H., Ignell, R. (2016). Chicken volatiles repel host-seeking malaria mosquitoes. *Malar. J.* 15, 354.

Kaburi, J.C., Githuto, J.N., Muthami, L., Ngure, P.K., Mueke, J.M. and Mwandawiro, C.S. (2009). Effects of long-lasting insecticidal nets and zooprophylaxis on mosquito feeding behavior and density in Mwea, central Kenya. *J. Vector Borne Dis.* 46, 184-190.

Karunamoorthi, K. (2011). Vector control: a cornerstone in the malaria elimination campaign. *Clin. Microbiol. Infect.* 17, 1608-1616.

Katz, T.M., Miller, J.H. and Hebert, A.A. (2014). Insect repellents: historical perspectives and new developments. *J. Am. Acad. Dermatol.* 58, 865-71.

Kent, J.R., Thuma, E.P., Mharakurwa, S. and Norris, E.D. (2007). Seasonality, blood feeding behavior and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in Southern Zambia. *Am. J. Trop. Med. Hyg.* 76, 267-274.

Killeen, F.G. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malar. J.* 13, 330.

Killeen, F.G. and Smith, A.T. (2007). Exploring the contributions of bed nets, cattle, insecticides and excitorepellency to malaria control: a deterministic model of mosquito host-seeking behaviour and mortality. *Trans. Roy. Soc. Trop. Med. Hyg.* 101, 867-880.

Killeen, G.F., Kihonda, J., Lyimo E., Oketch, F.R., Kotas, M.E., Mathenge, E., Schellenberg, J. A., Lengeler, C., Smith, T. A. and Drakeley, C. J. (2006). Quantifying behavioral interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. *BMC Infect Dis.* 6, 161

Killeen, G.F., McKenzie, F.E., Foy, B.D., Bogh, C., and Beier, J.C. (2001). The availability of potential hosts as a determinant of feeding behaviors and malaria transmission by African populations. *Trans. R. Soc. Trop. Med. Hyg.* 95, 469–476.

Killeen, G.F., Seyoum, A., Gimnig, J.E., Stevenson, J.C., Drakeley, C.J. and Chitnis, N. (2014). Made-to-measure malaria vector control strategies: rational design based on insecticide properties and coverage of blood resources for mosquitoes. *Malar. J.* 13,146.

Koudou, G.B., Koffi, A.A., Malone, D. and Hemingway, J. (2011). Efficacy of permaNet® 2.0 and permaNet® 3.0 against insecticide-resistant *Anopheles gambiae* in experimental huts in Côte d'Ivoire. *Malar. J.* 10, 172.

Lefèvre, T., Gouagna, L-C., Dabiré, K.R., Elguero, E., Fontenille, D., Renaud, F., Costantini, C. and Thomas F. (2009). Beyond Nature and Nurture: Phenotypic Plasticity in Blood-Feeding Behavior of *Anopheles gambiae* s.s. When Humans Are Not Readily Accessible. *Am. J. Trop. Med. Hyg.* 81, 1023–1029.

Lyimo, I.N., Ng'habi, K.R., Mpingwa, M.W., Daraja, A.A., Mwasheshe, D.D., Nchimbi, N.S., Dickson, W., Lwetoijera, D.W. and Mnyone, L.L. (2012). Does cattle milieu provide a

potential point to target wild exophilic *anopheles arabiensis* (Diptera: Culicidae) with entomopathogenic fungus? A bioinsecticide zooprophylaxis strategy for vector control. *J. Parasitol. Res.* doi:10.1155/2012/280583.

Mahande, A., Mosha, F., Mahande, J. and Kweka, E. (2007a). Feeding and resting behavior of malaria vector, *Anopheles arabiensis* with reference to zooprophylaxis. *Malar. J.* 6, 100.

Mahande, A., Mosha, F.W., Mahande, J.M. and Kweka, E.J. (2007b). Role of cattle treated with deltamethrine in areas with a high population of *Anopheles arabiensis* in Moshi, northern Tanzania. *Malar. J.* 6, 109.

Martens, P. and Hall, L. (2000). Malaria on the move: human population movement and malaria transmission. *Emerg. Infect. Dis.* 6, 103-109.

Massebo, F., Balkew, M., Gebre-Michael, T. and Lindtjørn, B. (2015). Zoophagic behavior of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasites Vectors* 8, 645.

Maxwell, C.A., Wakibara, J., Tho, S. and Curtis, C. F. (1998). Malaria-infective biting at different hours of the night. *Med. Vet. Entomol.*12, 325-7.

Mazigo, H.D., Obasy, E., Mauka, W., Manyiri, P., Zinga, M., Kweka, E.J., Mnyone, L.L., and Heukelbach, J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. *Malar. Res. Treat. 2010*, 794261

McGready, R., Simpson, J.A., Htway, M., White, N.L., Nosten, F. and Lindsay, S.W. (2001). A double-blind randomized therapeutic trial of insect repellents for the prevention of malaria in pregnancy. *Trans. R. Soc. Trop. Med. Hyg.* 95, 137-38.

Mueller, I., Galinski, M.R., Baird, J.K., Carlton, J.M., Kochar, D.K., Alonso, P.L. and del Portillo H.A. (2009). Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet Infect. Dis*. 9, 555-566.

N'Guessan, R., Corbel, V., Akogbeto, M. and Rowland, M. (2007). Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg. Infect. Dis.* 13, 199-206.

Najera, J.A., Gonza´lez-Silva, M. and Alonso, P.L. (2011). Some lessons for the future from the global malaria eradication program (1955–1969). *PLoS Med* 8, e1000412.

Newby, G., Bennett, A., Larson, E., Cotter, C., Shretta, R., Phillips, A.A. and Feachem, R.G.A. (2016). The path to eradication: a progress report on the malaria-eliminating countries. *Lancet* 387, 1775-1784.

Ngufor, C., N'Guessan, R., Boko, P., Odjo, A., Vigninou, E., Asidi, A., Akogbeto, M. and Rowland, M. (2011). Combining indoor residual spraying with chlorfenapyr and long-lasting

insecticidal bed nets for improved control of pyrethroid-resistant *Anopheles gambiae*: an experimental hut trial in Benin. *Malar. J.* 10, 343.

Palsson, K., Thomas, G.T., Dias, J.F., Laugen, A.T. and Bjorkman, A. (2004). Endophilic *Anopheles* mosquitoes in Guinea Bissau, west Africa, in relation to human housing conditions. *J. Med. Entomol.* 41, 746-752.

Pates, H. and Curtis, C. (2005). Mosquito behavior and vector control. *Annu. Rev. Entomol.* 50, 53-70.

Reddy, M.R., Overgaard, H.J., Abaga, S., Reddy, V.P., Caccone, A., Kiszewski, A.E. and Slotman M.A. (2011). Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malar. J.* 10,184.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J. and Fayaz, M. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop. Med. Int. Health* 9, 335-342.

Rowland, M., Downey, G., Rab, A., Tim Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C. and Lines, J. (2004). DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. *Trop. Med. Int. Health* 9, 335-42.

Rowland, M., Durrani, N., Kenward, M., Mohammed, N., Urahman, H. and Hewitt, S. (2001). Control of malaria in Pakistan by applying deltamethrin insecticide to cattle: a community-randomized trial. *Lancet* 357, 1837-1841.

Sangoro, O., Lweitojera, D., Simfukwe, E., Ngonyani, H., Mbeyela, E., Lugiko, D., Kihonda, J., Maia, M. and Moore, S. (2014a). Use of a semi-field system to evaluate the efficacy of topical repellents under user conditions provides a disease exposure free technique comparable with field data. *Malar. J.* 13**,** 159.

Sangoro, O., Turner, E., Simfukwe, E., Miller, J.E. and Moore, S.J. (2014b). A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long- lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission. *Malar. J.* 13, 324.

Sangoro, O., Turner, E., Simfukwe, E., Miller, J.E., Moore, S.J. (2014b). A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission. *Malar. J.* 13, 324.

Shililu, J., Ghebremeskel, T., Seulu, F., Mengistu, S., Fekadu, H., Zerom, M., Asmelash, G., Sintasath, D., Mbogo, C., Githure, J., Brantly, E., Beier, J. and Novak, R. (2004). Seasonal abundance, vector behaviour, and malaria parasite transmission in Eritrea. *J. Am. Mosq. Cont. Assoc.* 20, 155-64.

Sluydts, V., Durnez, L., Heng, S., Gryseels, C., Canier, L., Kim, S., Van Roey, K., Kerkhof, K., Khim, N., Mao, S., Uk, S., Sovannaroth, S., Grietens, K.P., Sochantha, T., Menard, D. and Coosemans, M. (2016). Efficacy of topical mosquito repellent (picaridin) plus long-lasting insecticidal nets versus long-lasting insecticidal nets alone for control of malaria: a cluster randomized controlled trial. *Lancet Infect. Dis.* 16, 1169-1177.

Takken, W. and Verhulst, N.O. (2013). Host Preferences of Blood-Feeding Mosquitoes. *Annu. Rev. Entomol.* 58, 433–53.

Taye, B., Lelisa, K., Emana, D., Asale, A. and Yewhalaw, D. (2016). Seasonal dynamics, longevity, and biting activity of anopheline mosquitoes in southwestern Ethiopia. *J. Insect Sci.* 6, 1-7.

Temu, E.A., Coleman, M., Abilio, A.P. and Kleinschmidt I. (2012). High prevalence of malaria in Zambezia, Mozambique: the protective effect of IRS versus increased risks due to pig-keeping and house construction. *PLoS One* 7, e31409.

Tirados, I., Costantini, C., Gibson, G. and Torr, S.J. (2006). Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Med. Vet. Entomol.* 20, 425-437.

Tungu, P., Magesa, S., Maxwell, C., Malima, R., Masue, D., Sudi, W., Myamba, J., Pigeon, O., and Rowland, M. (2010). Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against *Anopheles gambiae* and pyrethroid resistant *Culex quinquefasciatus* mosquitoes: an experimental hut trial in Tanzania. *Malar. J.* 9, 21.

USAID-PMI. (2016). Malaria operational plan FY 2016 - Ethiopia. President's malaria initiative, USAID. [online]. Available from: fy16/fy-2016-ethiopia-malaria-operational-plan.pdf [accessed on: 29/09/2016]

Van Bortel, W., Chinh, D.V., Berkvens, D., Speybroeck, N., Trung, D.H. and Coosemans, M. (2009). Impact of insecticide-treated nets on wild pyrethroid resistant *Anopheles epiroticus* population from southern Vietnam tested in experimental huts. *Malar. J.* 8, 248.

Waka, M., Hopkins, R.J., Akinpelu, O. and Curtis, C. (2005). Transmission of malaria in the Tesseney area of Eritrea: parasite prevalence in children, and vector density, host preferences, and sporozoite rate. *J. Vector Ecol.* 30, 27-31.

Weissmann, G. (2006). DDT is back: let us spray! *Faseb J.* 20, 2427-2429.

WHO (2015b). Indoor residual spraying: An operational manual for IRS for malaria transmission, control and elimination. 2nd ed. . [Online] Available from. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed: 12/30/2016]. [accessed: 04/04/2016].

WHO. (1969). Re-examination of the global strategy of malaria eradication. *Thirteenth plenary meeting, 24 July 1969 (Committee on Programme and Budget, fourth report).* Geneva: World health organization.

WHO. (1982). *Manual on environmental management for mosquito control with special emphasis on malaria vectors*. World health organization. Geneva, Switzerland

WHO. (2004). *Insecticide-treated bed nets and curtains for preventing malaria.* Geneva: World health organization. [Online] Available from:

WHO. (2006). *Pesticides and their application for the vectors and pests of public health importance.* Geneva: World health organization. Available at: WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf [accessed on: 09/16/2016]

WHO. (2013). *Larval Source management: a supplementary measure for malaria vector control: An Operational Manual.* [Online] Available from http://apps.who.int/iris/bitstream/10665/85379/1/9789241505604_eng.pdf [accessed on: 09/12/2016]

WHO. (2015a). World malaria report. World health organization, Geneva. [Online] Available from http://who.int/malaria/publications/world-malaria-report-2015/report/en/ [accessed: 04/04/2016].

WHO. (2016). *Eliminating malaria*: World health organization. [Online] Available from: WHO/HTM/GMP/2016.3. [accessed on: 11/7/2016]

Wilson, A.L., Chen-Hussey, V., Logan, J.G. and Lindsay, S.W. (2014). Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. *Malar J* 13, 446.

Yamamoto, S.S., Louis, V.R., Sie, A. and Sauerborn, R. (2009). The effects of zooprophylaxis and other mosquito control measures against malaria in Nouna, Burkina Faso. *Malar. J.* 8, 283.

Yewhalaw, D., Asale, A., Tushune, K., Getachew, Y., Duchateau, L. and Speybroeck, N. (2012). Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant *Anopheles arabiensis* from South-Western Ethiopia. *Parasites Vectors* 5, 159.

Yewhalaw, D., Van Bortel, W., Denis, L., Coosemans, M., Duchateau, L. and Speybroeck, N. (2010). First evidence of high knockdown resistance frequency in *Anopheles arabiensis* (Diptera: Culicidae) from Ethiopia. *Am. J. Trop. Med. Hyg.* 83, 122-125.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Bortel, W., Denis, L., Tessema, D.A., Getachew, Y., Coosemans, M., Duchateau, L. and Speybroeck, N. (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. *PLoS One* 6, 1.

Yohannes, M. and Boelee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Med. Vet. Entomol.*26, 103-105.

Summary

Summary

As a result of the scaling up of both LLIN and IRS the global malaria burden decreased substantially in the last decade. However, recent studies on the current malaria vector control methods showed that the efficacy of both LLINs and IRS could be potentially compromised due to the presence of insecticide resistance in the vector population. Furthermore, both LLINs and IRS are designed to provide protection against indoor biting mosquitoes, and thus residual transmission may continue even with complete LLINs coverage. Thus complementary tools should be put in place in order to sustain the gains obtained from LLINs/IRS and move towards the envisaged goal of malaria elimination.

This dissertation is composed of a literature review and experimental work. The general introduction (Chapter 1) consists of a literature review on malaria epidemiology, malaria vector bionomics and vector control. In the section which introduces malaria epidemiology we presented the brief overview of global malaria distribution with emphasis on malaria transmission in Sub-Saharan Africa. Review of disease burden was followed by description of malaria vectors and vector bionomics. In this section, the main vector species responsible for malaria transmission in different parts of world were reviewed. Both physico-chemical and biological factors that contribute to the vector population dynamics were also presented. This chapter also introduces background information on currently available vector control tools including long-lasting insecticidal nets, indoor residual spraying, environmental management, repellents and others.

In chapter 3, literature work on zooprophylaxis as an alternative malaria control strategy for *An. arabiensis* was reviewed. In this section, first the basic biology and taxonomy of *An. arabiensis* was presented. The resting and feeding behavior of *An. arabiensis* were explained. The host preference and biting activity of *An. arabiensis* was also reviewed. Previous research works on zooprophylaxis as supportive (show efficiency of zooprophylaxis in malaria vector control) and contradictory (introduction of zooprophylaxis could risk an increase in malaria incidence) were summarized. Furthermore, other confounding factors that need due consideration in the implementation of zooprophylaxis such as the specific vector species and vector behavior, the distance of livestock from human quarters, the socio-economic status of community were discussed.

The experimental work covered the assessment of the efficacy of the current malaria vector control interventions PermaNet 2.0® (LLINs) and DDT (IRS) (Chapter 4), assessing the host

preference of *An. arabiensis* (Diptera: Culicidae) (Chapter 5) and the combined effect of repellent and zooprophylaxis (Chapter 6).

Despite the high coverage of IRS and scaling up of LLINs, there is no documented information yet on the effect of insecticide resistance on the existing malaria vector control interventions in Ethiopia. Thus, the objective of Chapter 4 was to assess the impact of insecticide resistance on malaria vector control interventions (LLINs and IRS) in Ethiopia. We evaluated the efficacy of both IRS (DDT) and LLINs (PermaNet 2.0) using field experimental huts. In our experiments we purposefully made holes in the nets (both LLINs and untreated nets) to assess whether the coated insecticide and not the net itself was effectively preventing mosquito bites. For IRS evaluation, we compared the proportion of fed mosquitoes (as compared to total number of mosquitoes collected) in DDT sprayed hut and unsprayed hut. There was no significant difference ($p > 0.05$) in mosquito blood feeding rates between sprayed (76.1%) and unsprayed hut (80.3%) and between a hut with treated net (55.1%) and the hut with untreated net (58.9%). Moreover, the mean exit rate was similar ($P > 0.05$) for sprayed hut (48.6%) and unsprayed hut (42.3%) and between a hut with treated net (49.4%) and a hut with untreated net (41.4%). There was no significant ($P > 0.05$) difference in mosquito mortality between sprayed and unsprayed hut nor between a hut with LLIN and a hut with untreated net. Thus, the results from Chapter 4 showed that the vector mosquito population from southwestern Ethiopia developed resistance which may jeopardize the current intervention tools. The origin of insecticide resistance mechanism can be point mutation, metabolic resistance or behavioral resistance. The later can be displayed in the form of shifting resting places, changing or alternating among different blood meal source hosts, shifting time of biting or can be combination of the above. Therefore, in chapter 5 the host preference of *An. arabiensis* was assessed using three alternative experimental setups in Southwestern Ethiopia.

The results of the three different host preference experiments showed that populations of *An. arabiensis* from Jimma area were zoophagic. In the first experimental set up of the study, the density of *An. arabiensis* was significantly higher in the enclosure trap with calf ($P < 0.001$) as compared to the enclosure trap with human. However, the density of *An. arabiensis* was significantly lower in the enclosure trap with chicken ($P = 0.002$) and goat ($P < 0.001$) as compared to the enclosure trap with human. In the second experiment, the density of *An. arabiensis* was significantly higher in the enclosure trap with calf ($P < 0.001$) and goat ($P < 0.001$) as compared to the enclosure trap with human. Similarly, identification of blood meal source has shown also that a significantly higher density of *An. arabiensis* mosquitoes fed on calf ($P < 0.001$) as compared to human.

In Chapter 6 we evaluated the additive effect of repellent and zooprophylaxis in malaria vector control in Southwestern Ethiopia. In this study the efficacy of Buzz off (petroleum jelly, essential oil blend), Mozigone (plant derived essential oil blend) and DEET, a standard repellent, were assessed using arm-in-cage experiment and semi-field setup using human volunteers applying repellent as a push factor and a calf as pulling factor. The median complete protection time for Buzz off, Mozigone, and DEET using wild populations of *An. arabiensis* was 3, 60 and 300 minutes, respectively. Significantly higher mosquito density was recorded in enclosure traps without repellent as compared to enclosure traps containing human volunteers applying Mozigone (mean difference = 15.25; $P < 0.001$), Buzz off (mean difference = 6.25; $P = 0.045$) and DEET (mean difference = 9.75; $P = 0.008$). Similarly, significantly higher mosquito density was recorded from enclosure traps containing calf as compared to human volunteers using Mozigone (mean difference = 11.75; $P = 0.027$) and DEET (mean difference = 18.75; $P = 0.004$), but not for Buzz off.

In conclusion, the evaluation of IRS using DDT and LLINs (PermaNet® 2.0) based on a trial using experimental huts suggests that neither DDT nor LLIN can stand alone as a vector control tool in the presence of the resistant mosquito population in the region. Therefore, alternative new vector control tools should be put in place and an insecticide resistance management strategy should be developed and implemented. Furthermore, large scale field trials should be carried out in order to confirm whether the current vector control interventions, IRS and LLINs, are still effective in different regions of Ethiopia. We showed that cattle may play a potential role as a barrier or effectively divert malaria vectors from human to livestock hosts. Other complementary factors, such as the use of insecticides on the livestock host and the optimal distance between livestock enclosure and the human dwellings need to be further investigated. Mozigone (plant based essential oil) provided relatively better protection as compared to Buzz off and its bio-prospective aspects should be further examined using field trial. Both Buzz off and Mozigone are short lived repellents. Thus, DEET remains the reliable personal protection currently available. Zooprophylaxis should be evaluated in a local-specific approach as in some countries it is effective whereas in others not. Future studies on estimation of the distance threshold between human quarters and livestock pen, the additive effect of cluster randomized field trial on repellent and zooprophylaxis could further strengthen the efficacy of zooprophylaxis.

Samenvatting

Samenvatting

Malaria prevalentie is in de laatste 10 jaar sterk afgenomen, voornamelijk dankzij twee interventies die de malariamug bestrijden, nl. het gebruik van met insecticide behandelde bednetten (LLIN) en het sproeien van insecticiden in het huis of de hut (IRS). Recent werd evenwel vastgesteld dat de doeltreffendheid van deze twee interventies vermindert doordat de vectorpopulatie resistent wordt tegen de meest gebruikte insecticiden. De twee interventies, LLIN en IRS, zijn ook voornamelijk gericht op malariamuggen die binnenshuis voeden en bijgevolg zal beperkte malariatransmissie blijven optreden, ook indien de hele populatie bednetten zou gebruiken. Het is bijgevolg noodzakelijk om complementaire interventietechnieken te ontwikkelen naast LLIN en IRS, zodat op termijn malaria kan uitgeroeid worden.

Deze dissertatie bestaat enerzijds uit literatuuronderzoek en anderzijds uit eigen werk.

De algemene inleiding (Hoofdstuk 1) bestaat uit een overzicht van de epidemiologie van malaria, een bespreking van de vector species en de bestaande vector controlemethoden. Er wordt een kort overzicht gegeven van de globale malaria verdeling met de nadruk op Sub-Sahara Afrika, gevolgd door een beschrijving van de malaria vector en de vector bionomics. De dominante vector species die wereldwijd verantwoordelijk zijn voor malaria transmissie worden besproken. Tevens worden zowel biologische als fysische factoren die een rol spelen in de vector populatie dynamiek voorgesteld. Tenslotte worden ook de verschillende meest gebruikte vector controletechnieken besproken, met name met insecticide behandelde bed netten (LLIN), het sproeien van insecticiden in het huis of de hut (IRS), management van de omgeving en het gebruik van afweermiddelen tegen insecten.

In Hoofdstuk 3 wordt de literatuur in verband met zoo-profylaxe als een alternatieve malaria controle strategie voor *An. arabiensis* samengevat. De taxonomie en basisbiologie van *An. arabiensis* wordt besproken, met de nadruk op het rust- en voedingsgedrag, de voorkeur voor bepaalde gastheren en de bijtactiviteit. Gepubliceerde resultaten ondersteunen ofwel het gebruik van zoo-profylaxe, i.e., door zoo-profylaxe wordt de malariavector gecontroleerd, of spreken dit tegen, i.e., de introductie van zoo-profylaxe heeft een verhoging van het voorkomen van malaria tot gevolg. Tenslotte volgt er een discussie over andere factoren die een invloed hebben op het al dan niet succesvol toepassen van zoo-profylaxe, zoals de specifieke vector species en het bijhorende gedrag, de afstand tussen de dieren en de slaapplaats van het gezin en de socio-economische status van de gemeenschap.

In het experimentele werk werd de doeltreffendheid van de huidige malaria vector controle interventies PermaNet 2.0® (LLINs) en DDT (IRS) nagegaan (Hoofdstuk 4), werd de

gastheerpreferentie van *An. arabiensis* (Diptera: Culicidae) bepaald (Hoofdstuk 5) en het gecombineerde effect van afweermiddelen tegen insecten en zoo-profylaxe bestudeerd (Hoofdstuk 6).

Er bestaan weinig tot geen studies over de invloed van insecticide resistentie op de meest gebruikte malaria vector controle interventies in Ethiopië, IRS en LLINs. Daarom werd in Hoofdstuk 4 de impact van insecticide resistentie op de malaria vector controle interventies LLINs en IRS in Ethiopië bestudeerd. De doeltreffendheid van zowel IRS (DDT) en LLINs (PermaNet 2.0) werd geëvalueerd waarbij gebruik gemaakt werd van experimentele hutten in het veld. Er werden gaten gemaakt in de bed-netten (zowel de LLINs als de onbehandelde netten) zodat eerder het effect van het gecoate insecticide dan wel de fysische barrière van het net getest werd om muggenbeten te voorkomen.

Voor de evaluatie van IRS werd de proportie gevoede muggen (ten opzichte van het totaal aantal verzamelde muggen) in DDT gesproeide en niet-gesproeide hutten bepaald. Er was geen significant verschil ($p > 0.05$) tussen de gesproeide (76.1%) en niet-gesproeide hut (80.3%) of tussen een hut met een behandeld net (55.1%) en een onbehandeld net (58.9%). Bovendien was de gemiddelde 'exit rate' gelijkaardig ($P > 0.05$) voor de gesproeide hut (48.6%) en niet-gesproeide hut (42.3%) en voor een hut met een behandeld net (49.4%) en een onbehandeld net (41.4%). Ook voor de mortaliteit van de muggen was er geen significant verschil ($P > 0.05$) tussen de gesproeide en niet-gesproeide hut of tussen de hut met een behandeld en onbehandeld bed net. Op basis van deze resultaten kunnen we concluderen dat de vectorpopulatie in zuidwest Ethiopië resistentie heeft ontwikkeld tegen de gebruikte insecticiden waardoor deze interventie ineffectief is geworden. Daarom is het noodzakelijk om dringend nieuwe vector controle strategieën te ontwikkelen en te implementeren. Een mogelijke interventie is gebaseerd op zoo-profylaxe, eventueel gecombineerd met andere ondersteunende maatregelen. Het potentieel van zoo-profylaxe in zuidwest Ethiopië werd verder onderzocht in deze dissertatie.

De gastheerpreferentie van *An. arabiensis* (Diptera: Culicidae) werd bestudeerd in zuidwest Ethiopië en de resultaten werden beschreven in Hoofdstuk 5. Er werden drie alternatieve experimentele proefopzetten gebruikt. De resultaten van de drie verschillende experimentele proefopzetten toonden aan dat *An. arabiensis* populaties van de Jimma regio voorkeur vertoonden om te voeden op koeien maar aan de andere kant eerder de mens dan kippen verkozen. In het eerste experiment met de natuurlijke muggenpopulatie, i.e., muggen komen

de experimentele hutten binnen vanuit de omgeving, was het aantal *An. arabiensis* muggen significant hoger in het compartiment met een kalf ($P < 0.001$) in vergelijking met het compartiment met een persoon. Het aantal *An. arabiensis* muggen was daarentegen significant lager in het compartiment met kippen ($P = 0.002$) en een geit ($P < 0.001$) in vergelijking met het compartiment met een persoon. In het tweede experiment met een gecontroleerde muggenpopulatie, i.e., laboratoriummuggen worden vrijgelaten in de experimentele hutten, was het aantal *An. arabiensis* muggen significant hoger in het compartiment met een kalf ($P < 0.001$) en een geit ($P < 0.001$) in vergelijking met het compartiment met een persoon. Soortgelijk voedde een significant hoger aantal *An. arabiensis* muggen op kalf in vergelijking met een persoon.

In Hoofdstuk 6 werd het additieve effect van afweermiddelen en zoo-profylaxe in malaria vector controle in zuidwest Ethiopië bestudeerd. De doeltreffendheid van Buzz off (petroleumgelei bestaande uit een mengsel van essentiële oliën), Mozigone (een plantaardig mengsel van essentiële oliën) en DEET (een standaard afweermiddel) werd bepaald aan de hand van arm-in-kooi experimenten en in semi-veld experimenten met vrijwilligers die het afweermiddel gebruikten en kalf als een factor om muggen aan te trekken. De mediane volledige beschermingstijd voor Buzz off, Mozigone en DEET gebaseerd op een veldpopulatie van *An. arabiensis* bedroeg resp. 3, 61 en 302 minuten. Een significant hoger aantal muggen werd opgemeten in de compartimenten met personen zonder afweermiddel in vergelijking met de compartimenten met personen met afweermiddel Mozigone (gemiddeld verschil = 15.25; $P < 0.001$), Buzz off (gemiddeld verschil = 6.25; $P = 0.045$) en DEET (gemiddeld verschil = 9.75; $P = 0.008$). Soortgelijk werd een significant hoger aantal muggen opgemeten in de compartimenten met een kalf in vergelijking met de compartimenten met personen met afweermiddel Mozigone (gemiddeld verschil = 11.75; $P = 0.027$) en DEET (gemiddeld verschil = 18.75; $P = 0.004$), maar niet voor Buzz off.

Gebaseerd op de studies waarin IRS met DDT en LLINs (PermaNet® 2.0) werd toegepast in experimentele hutten kunnen we besluiten dat geen van beide interventies nog effectief is in de studieregio doordat de muggenpopulatie resistentie heeft opgebouwd tegen de insecticiden waarop deze interventies gebaseerd zijn. Het is bijgevolg essentieel dat alternatieve nieuwe vector controle interventies worden ontwikkeld en geïmplementeerd en dat een strategisch plan voor het management van insecticide resistentie wordt ontwikkeld en uitgevoerd. Er dienen verder grote veldstudies opgezet te worden in verschillende regio's in Ethiopië om te evalueren of de efficiëntie van IRS en LLINs in het hele land vermindert. We toonden verder aan dat runderen kunnen gebruikt worden om de malariamuggen af te leiden van de mens. Andere

factoren, zoals het gebruik van insecticide op runderen en de optimale afstand tussen het rund en de mens dienen verder onderzocht te worden. Mozigone (een plantaardig mengsel van essentiële oliën) gaf betere bescherming dan Buzz off, en zou verder moeten bestudeerd worden in veldexperimenten. Zowel Buzz off als Mozigone zijn afweermiddelen die slechts een beperkte activiteit hebben in de tijd. DEET blijft het enige betrouwbare persoonlijke beschermingsmiddel dat beschikbaar is in Ethiopië. Het effect van zoo-profylaxe moet steeds opnieuw geëvalueerd worden in specifieke regio's; in sommige regio's werd het positieve effect bewezen, in andere regio's net het tegendeel. In regio's waar zoo-profylaxe een positief effect heeft, kan deze interventietechniek verder geoptimaliseerd worden. Mogelijke verbeteringen bestaan uit het optimaliseren van de afstand tussen de verblijfplaats van de mens en het dier, en het combineren van zoo-profylaxe met het gebruik van afweermiddelen.

ማጠቃለያ

ማጠቃለያ

የአልጋ አጎበሮችንና የቤትለቤት ርጭትን በከፍተኛ ደረጃ ማዳረሰና ማስፋትን ተከትሎ የወባ በሽታ ተፅዕኖ ባለፉት አስርት ዓመታት ዉስጥ በእጅጉ ቀንሷል፡፡ ሆኖም ግን ከቅርብ ጊዜ ወድህ እየወጡ ያሉ የምርምር መረጃዎች እንደሚያሳዩት ከሆነ የወባ በሽታ አስተላላፊ ትንኞች ፀረ-ነፍሳት ኬሚካሎችን በተለይም ለአጎበር መንከሪያና ለቤት ርጭት የሚዉሉ ከሚካልን እየተላማመዱና እየተቋቋሙ መምጣታቸዉ አጠቃላይ በሽታዉን ለመቆጣጠር የሚደረገዉን ሁለ-ገብ ጥረት ወደኋላ እንዳይጎትተዉ ይፈራል፡፡ ከዚህም በተጨማሪ ሁለቱም የወባ ትንኝ መቆጣጠሪያ ዘዴዎች (አጎበርና የቤትለቤት ርጭት) በዋናነት ሰዎች በቤታቸዉ ዉስጥ በሚሆኑበት ጊዜ በሽታ ተሸካሚ ትንኞች ወደ ቤት ግብተዉ አንድም በግድግዳ ላይ እንዳያርፉ፤ ቀጥሎም ከሰዉ ጋር ንክኪ እንዳይኖራቸዉ በማድረግ በሽታዉ እንዳይዛመት የሚደረግ ቤት-ወስጥ-ተኮር ስትራቴጂ ብቻ መሆናቸዉ የየራሳቸዉ ዉስንነት እንድኖራቸዉ አድርጓል፡፡ ይህ ማለት አጎበርና የቤትለቤት ርጭትን መቶ በመቶ ማዳረስ ብቻል እንኳን ካለባቸዉ ዉስንነት የተነሳ የበሽታዉን ስርጭት ሙሉ በሙሉ ለማቋረጥ እጅግ አስቸጋሪ ያደርገዋል፡፡ ምክኒያቱም ከቤት ዉጭ ከወባ ትንኝ ጋር በሚኖረን ንክኪ ምክንያት የበሽታዉ ስርጭት ስለሚቀጥል በሚፈለገዉ ደረጃ የበሽታዉን ስርጭት መቀነስ አስቸጋሪ ይሆናል፡፡ በመሆኑም የታለመዉን የወባ በሽታን ከአገራችን የማጥፋት ግብ ለማሳካት ሌሎች ተደጋጋፊና ተጨማሪ የወባ ትንኝ መቆጣጠሪያ ዘዴዎች(መሳሪዎች) ያሰፈልጋሉ፡፡

ይህ የምረቃ ምርምር ሁለት ዋና ዋና ክፍሎች አሉት፤የመጀመሪያዉ ክፍል በወባ በሽታና አስተላላፊ የወባ ትንኞች ዙሪያ የተጠናቀረ ቅደመ-ምርምር ዳሰሳ ስሆን ሁለተኛዉ የቤቴ-ሙከራና የመስክ ምርምር ዉጤቶችን የሚንተነትንበት ክፍል ነዉ፡፡

በመጀመሪያ ምዕራፍ (ምዕራፍ ፩) አጠቃላይ መግቢያ ዳሰሳ ተደርጓል፡፡ በዚሁ መሰረት የወባ በሽታ ችግር ስርጭትና ስፋት፣ የወባ በሽታ ትንኞች ዝሪያቸዉ እና አስተላላፊ ትንኞች መቆጣጠሪያ ስልቶች ተዳስሷል፡፡ የወባ በሽታ ችግር ስርጭትና ስፋትን በተመለከተ አጠቃላይ አለም-አቀፋዊ ገጽታ በተለይም በአፍሪካና በኢትዮጵያ ትኩረት በማድረግ ተተንትኗል፡፡ በማሰከተል የወባ ትንኞች፣ ዝሪያቸዉ፤ስርጭታቸዉና፣ኑሮ-ዜዴያቸዉ ተዘርዝሯል፡፡ በተለይም ደግሞ ከወባ ትንኞች መካከል አዉራ አስተላላፊ ዝሪያዎች ለሆኑት ልዩ ትኩረት በመስጠት ዳሰሳ ተደርጓል፡፡ ከዚህ ጋር በማያያዝ ለወባ ስርጭትና ለትንኞች ቁጥር ከፍ-ዝቅ ፍሰት ምክንያት የሚሆኑ ስነ-ሕይወታዊና ቁሳዊ ሁኔታዎች ተዘርዝሯል፡፡ የወባ ትንኞችን ለመቆጣጠር የሚንጠቅምባቸዉ መሳሪያዎች እንደ አልጋ አጎበር፣ የቤትለቤት ርጭት፣የወባ ትንኝ መራቢያ አከባቢን ማንጠፍና አርቂ ከሚካልን መጠቀምን በተመለከተ ዳሰሳ ቀርቧል፡፡

በምዕራፍ ፫ የወባ ትንኞችን (*Anopheles arabiensis)*ን ለመቆጣጠር (zooprophylaxis) (የቤት እንስሳት ከለላ) እንደአማራጭ መፍትሄ በሚል ርዕሰ-ጉዳይ ጥናታዊ ዳሰሳ ተደርጓል፡፡ በዚሁ ጥናታዊ ዳሰሳ ስር የወባ አስተላላፊ የሆኑ ትንኞች መሰረታዊ ስነ-ሕይወትና ሳይንሳዊ የትንኞች መለያ ዜዴ ቀርቧል፡፡ በተለይም ደግሞ ጠለቅ ያለ የትንኞች የተፈጥሮ ባህሪን ለመረዳት የሚጠቅሙ ትንታኔዎች ለምሳሌ የወባ አስተላላፊ ትንኞች በምን ዓይነት ስፍራ ያርፈሉ? የትኞቹን እንስሳቶች ይመገባሉ? በሚመገቡበት ሰዓት ከተለያዩ እንስሳት፣ ከሰዉና ከእንስሳት የትኛዉን የበለጠ ይመርጣሉ? በምን ሰዓት ይነድፋሉ? በሚሉ ንዑስ-ርዕሶች ዙሪያ ጥናታዊ ዳሰሳ ተደርጓል፡፡ የቤት እንስሳት ከለላ (zooprophylaxis) በተመለከተ በአንድ በኩል ደጋፊ ምርምሮች በሌላ በኩል አሉታዊ ገጹን የሚያንሉ ምርምሮች ዳሰሳ ተደረጓል፡፡ በተጨማሪም ከቤት እንስሳት ከሌላ (zooprophylaxis) ጋር በቀጥታም ሆነ በተዘዋዋሪ የሚገናኙና ኢጋዥ ወይም አባባሽ ጉዳዮች ለምሳሌ የወባ ትንኝ ዝሪያ ዓይነት፣ የተፈጥሮ ባህሪያቸዉ፣ በሰዎች ማረፊያና በእነስሳት በረት መካከል ልኖር የሚገባዉ ርቀት፣ የሰዎች ማህበራዊና እኮኖሚያዊ ጉዳዮች ተዳስሷል፡፡

የቤቴ-ሙከራና የመስክ ምርምር ስራዎችን በተመለከተ፡- በምዕራፍ ፬ በጥናቱ ወቅት በጂማ አከባቢ በጥቅም እየዋሉ ያሉ የወባ ትንኝ መቆጣጠሪያ ዜዴዎች ማለትም የአልጋ አጎበር (PermaNet 2.0® (LLINs)ና የቤት ዉስጥ ርጭት (DDT (IRS) ያሉበት ወቅታዊ የመከላከል አቅም ሚዘና ተደረጓል፡፡ በምዕራፍ ፭ በአከባቢዉ የሚገኙ የወባ ትንኝ ዝሪያ (*Anopheles arabiensis*) የሚመገቡት የተለያዩ የእንስሳት ዓይነቶችና አንፃራዊ ምርጫቸዉ ተዳስሷል፡፡ በምዕራፍ ፮ አርቂ ከሚካልን ከእንስሳት ከለላ ጋር ማጣመር የወባ ትንኞችን ለመቆጣጠር ያለዉ ጠቀመታ ተገምግሟል፡፡

በተለመዶ የወባ ትንኞችን ለመቆጣጠር በሚደረገዉ እንቅስቃሴ ከፍተኛ ቁጥር ያለዉ የአልጋ አጎበርና መጠነ-ሰፊ የሆነ ቤት ዉስጥ ርጭት ይደረጋል፡፡ ሆኖም ግን ትንኞች ፀረ-ነፍሳት ከሚካልን በመቋቋማቸዉ ለመቆጣጠር የሚደረጉ ጥረቶች የተቀመጠላቸዉን ግብ ይምቱ ወይም አይምቱ በትክክል የተደረገ ግምገማ የለም፡፡ በመሆኑም የምዕራፍ ፬ ዋና ዓላማ የነበረዉ የወባ ትንኞች ፀረ-ነፍሳት ከሚካልን መቋቋም በኢትዮጵያ ዉስጥ በሚተገበሩ ትንኝ መቆጣጠሪያ ዜዴዎች (LLINs and IRS) ላይ የሚኖረዉ ተፅዕኖ መፈተሽ ነዉ፡፡ ይሄንንም ለማረጋገጥ እንድያመች አንድ ለርጭት የሚሆን ከሚካል (DDT) አንድ አልጋ አጎበር (PermaNet 2.0) በመዉሰድ ለዚሁ ተግባር ስባል በመስክ የተሰሩ አራት ቤቴ-መኪራ ጎጆዎችን በመጠቀም ምርምር ተደርጓል፡፡ የአልጋ አጎበር የተነከረበት ከሚካል ዓላማዉ ትንኞች አጎበርን አልፈዉ ሰዎችን ለመንደፍ ጥረት በሚያደርጉበት ጊዜ እንድመረዙና እንድሞቱ ወይም ከርቀት ወደ ሰዎች መኝታ እንዳይጠጉ በማራቅ እንድከላከል ታስቦ ነዉ፡፡ ይህ ማለት አጎበሩ በሰዉና በትንኞች መካከል እንደአጥር ሆኖ ከሚሰጠዉ ጥቅም በተጨማሪ ማለት ነዉ፡፡ ይህንን የኬሚካል ጥቅም ለመፈተሽ እንድረዳን አድስ አጎበር በመዉሰድ ትንንሽ ቀዳዳዎች እንድኖረዉ በማድረግ ሆን ተብለዉ በተፈጠሩ ቀዳዳዎች አመካይኝነት አጎበርን አልፈዉ የሚመገቡ ትንኞችን ዓይነትና ቁጥራቸዉን በመለካት፣ ለማለፍ ስሞከሩ የተመረዙና የሞቱ ትንኞችን በማስላት አጎበሩ የተነከረበት ከሚካል መስራት አለመስራቱን ለማወቅ ሚዘና ተደርጓል፡፡ ለቤት ዉስጥ ርጭት ከሚዉሉ ኬሚካሎች መካከል DDT በመጠቀም ሚዘና ተደርጓል፡፡ የምዘናዉን ዓላማና አጠቃላይ ህደት ለመቆጣጠር እንዲያመች ጎን ለጎን የሚከሄዱ ተመሳሳይ ሚዘናዎች ተደርጓል፡፡ የአጎበሩን ሚዘና ትክክለኛነት ለመቆጣጠር እንዲያመች ያልተነከረ ነገር ግን ተማሳሳይ ቁጥር ትንንሽ ቀዳዳዎች በሚኖረዉ አጎበር ዉስጥ ሰዉ እንድተኛ በማድረግ በተመሳሳይ መልኩ ሆን ተብለዉ በተፈጠሩ ቀዳዳዎች አመካይኝነት አጎበርን አልፈዉ የሚመገቡ ትንኞችን ዓይነትና ቁጥራቸዉን በማስላት አጎበሩ መስራት አለመስራቱን ለማወቅ ሚዘና ተደርጓል፡፡ በተመሳሳይ መልኩ DDT ላይ የተደረገዉ ሚዘና ትክክለኛነቱን ለመቆጣጠር እንዲያመች ባልተረጨ የመስክ ቤቴ-መኪራ ጎጆ ዉስጥ ሰዉ እንድተኛ በማድረግ ዉደ ጎጆ ዉስጥ የገቡትንና የተመገቡትን በተመሳሳይ መልኩ በተረጭ ጎጆ ዉስጥ ከገቡትና ከተመገቡት ጋር በማመሳከር ያለዉ ልዩነት ተፈትሿል፡፡

የዚህ ምርምር ዉጤት እንደሚያሳየዉ ከሆነ ትንኞች ወደተረጨ ቤት መግባት ብቻ ሳይሆን ከገቡት አጠቃላይ ትንኞች መከከል 76.1 ከመቶ የሚሆኑት ሰዎችን ነድፈዋል፤ ይህም ወዳልተረጨ ቤት ገብተዉ ሰዎችን ከነደፉት 80.3 ከመቶ ትንኞች ጋር ስነፃፀር ርጭቱ ትርጉም ባለዉ ደረጃ መጠነ-ንድፈትን እንዳልቀነሰ እንገነዘባልን (P > 0.05) ፡፡ አጎበርም ይሁን ርጭት ከሚሰጧቸዉ ጥቅሞች አንዱ የወባ ትንኞች ወደ ሰዎች ማረፊያ አከባቢ እንዳይቀርቡ ቀስ በቀስ በሚለቀቁ ትናኝ ከሚካሎች አማካይኝነት ትንኞችን ማራቅ ነዉ፡፡ ይህን ጥቅም ቤት ዉስጥ ከገቡ በኋላ ለቀዉ የሚወጡ ትንኞችን በማጥመድና በማስላት ማወቅ ይቻላል፡፡ ይህንን ጥቅም ለማስላት በተደረገዉ ጥረት ወደተረጨ ቤት ከገቡት አጠቃላይ ትንኞች መከከል 48.6 ከመቶ የሚሆኑት ለመዉጣት ስሞከሩ የተጠመዱ ሲሆን ይህም ወደልተረጨ ቤት ገብተዉ ለመዉጣት ስሞከሩ ከተጠመዱት 42.3 ከመቶ ጋር ስነፃፀር በተረጨ ቤት ዉስጥ ልኖር ከሚጠበቀዉ መጠነ-መዉጣት ምጣኔ እጂግ ያነሰና በተረጨና ባልተረጨ ቤት መካከል የተመዘገበዉ ምጣኔ ትርጉም ባለዉ ደረጃ አለመለያየቱን ነዉ (P > 0.05) ፡፡ በተመሳሳይ መልኩ የተነከረ አጎበር ቤት ከገቡት አጠቃላይ ትንኞች መከከል 49.4 ከመቶ የሚሆኑት

ለመዉጣት ስሞከሩ የተጠመዱ ሲሆን ይህም ያልተነከረ አጎበር ቤት ገብተዉ ለመዉጣት ስሞከሩ ከተጠመዱት 41.4 ከመቶ ጋር ስነፃፀር የተነከረ አጎበር ቤት ዉስጥ ልኖር ከሚጠበቀዉ መጠነ-መዉጣት ምጣኔ እጂግ ያነሰና ያልተነከረ አጎበር ቤት ዉስጥ ከተመዘገበዉ ምጣኔ ትርጉም ባለዉ ደረጃ አለመለያየቱን ነዉ (P > 0.05) ::

በመሆኑም ምዕራፍ ፬ ላይ ከተሰራዉ የምርምር ስራ የሚንደምድመዉ ዋና ነገር ይህ ጥናት በሚካሄድቤት ጊዜ ለወባ ትንኝ መቆጣጠሪያ በጥቅም ላይ እየዋሉ ያሉ የአልጋ አጎበሮች እና ርጭት ኬሚካል (DDT) የታቀደላቸዉን ያክል ዉጤታማ አለመሆናቸዉን ነዉ፤ለዝህም ምክንያቱ በተለይም በደቡብ ምዕራብ ኢትዮጵያ አከባቢ ያለዉ የወባ ትንኝ ዝሪያ ፀረ-ነፍሳት ኬሚካሎችን በመቋቋሙ ነዉ:: ከዘህ የሚንረዳዉ ሌሎች አዳድስና ተደጋጋፊ የትንኝ መቆጣጠሪያ ዜዴዎችን ማልማትና ስራ ላይ ማወል እንዳለብን የሚያመለክት ነዉ:: ከነዚህ ተደጋጋፊና አዳድስ መንገዶች አንዱ የቤት እንስሳት ከለላ (zooprophylaxis) ከሌሎች በስራ ላይ ካሉ ዜዴዎች ጋር በመቀመር መጠቀም ነዉ:: ስለዚህ የቤት እንስሳት ከለላ የወባ ትንኝን ለመካላከል ያለዉ አሰተዋፆ የዚህ ምርምር ማዕከላዊ ሀሳብና ማጠንጠኛ ነዉ::

የምዕራፍ ፭ ዋና ዓላማ በደቡብ ምዕራብ ኢትዮጵያ የሚገኙ የወባ ትንኝ ዝሪያዎች (*Anopheles arabiensis*, Diptera: Culicidae) መግቦተ-ምርጫን (Feeding preference) ወይም ተቀባዮች-ምርጫን (host preference) ለማጥናት ነዉ:: ይህ ጥናት የተካሄደዉ በጊልጌል ጊቤ የኤሌክተሪክ ሀይል ማመንጫ አከባቢ በተገነቡ አራት የመስክ ምርምር ጎጆዎችና በሶኮሩ የጂማ ዩኒቨርሲቲ ካምፓስ ዉስጥ በተገነባዉ የቲሮፒካልና ተላላፊ በሽታዎች መስክ-መለስ የጥናት ማዕከል ዉስጥ ስሆን የወባ ትንኝ የተፈጥሮ አመጋገብና የምግብ ምርጫቸዉ በሶስት አማራጭ የመስክ-መለስ ጥናት ዜዴዎች ተፈትሿል:: በሶስቱም የጥናት አማራጮች እንደተረጋገጠዉ በአካባቢዉ የሚገኙ የወባ ትንኝ ዝሪያ ሰዉና ከብትን በሚያገኝበት ጊዜ ከሰዉ ይልቅ ከብትን መመገብ እንደሚመርጥ በአንፃሩ ደግሞ ሰዉና ዶሮ በሚገኝበት ጊዜ ሰዉን መመገብ እንደምመርጥ ለማወቅ ተችሏል:: በመጀመሪያ የመስክ-መለስ ምርምር ማዕከል፤ በመስክ በተገነቡ ጎጆዎች ዉስጥ መለስተኛ መጠን ያላቸዉ ሁለት ዲንኳኖች የሚቀመጡ ስሆን በአንዱ ድነኳን ዉስጥ ሰዉ፣ በሌላኛዉ ድንኳን ዉስጥ የቤት እንስሳት፣ እንድቀመጡ ይደረግና ከዉጪ በርረዉ ወደ ጎጆ ዉስጥ የሚገቡ ትንኞች እንደምርጫቸዉ ወደ ሰዉ ድንኳን ወይም ወደ እነስሳት ድንኳን እንድገቡ የሚደረገበት ዜዴ ነዉ:: ቢዘሁ መሰረት የድንኳኑ አሰራር አንድ ጊዜ ወደዉስጥ የገቡ ትንኞች ወደዉጭ እንዳይመለሱ አድርጎ ስለሚጠምዳቸዉ አብዘኛዎቹ ትንኞች እንደየምርጫቸዉ በገቡበት ድንኳን ይቆዩና በሰተመጭረሻ አነጋግ ላይ ይለቀማሉ:: በዙሁ መሰረት ከፍተኛ ቁጥር ያለዉ ትንኝ ጥጃ ከሚቀመጥበት ድንኳን የተለቀመ ሲሆን ይሄም ቁጥር ሰዉ ከሚቀመጥበት ድንኳን ዉስጥ ከተለቀመዉ የትንኝ ቁጥር ጋር ስነፃፀር እጅግ የነላ ድርሻ ($P < 0.001$) እንዳለዉ ለማወቅ ተችሏል:: በሌላ በኩል ደግሞ ዶሮ ከተቀመጠበት ድንኳንና ፍየል ከተቀመጠበት ድንኳን የተለቀመዉን የትንኝ ቁጥር ሰዉ ከሚቀመጥበት ድንኳን ከተለቀመዉ የትንኝ ቁጥር ጋር በሚናነፃፀርበት ጊዜ በአብዘኛዉ ሰዉ ወደ ተቀመጠበት ድንኳን የሚሳብ መሆኑንና የጉልህነት መለያ መጠነ-መለከያቸዉም በዶሮና በሰዉ መካከል ($P = 0.002$) እና በፍየልና በሰዉ መካከል ($P < 0.001$) እንደሆነ ለማወቅ ተችሏል:: በሁለተኛዉ የመስክ-መለስ የጥናት ዜዴ ትንኞች ከወጭ መጥተዉ ወደ ድንኳን በመግባት ፋንታ ዝግ በሆነ ስስተም ዉስጥ በቁጥር የሚታወቅ በላብራቶሪ የተራቡ ትንኞች ይለቀቁና እንደየምርጫቸዉ ወደ ፈለጉት ድንኳን እነድገቡና እንድጠመዱ የሚደረግበት ዜዴ ስሆን በተመሳሳይ መልኩ በዘህም ጥናት እንደተረጋገጠዉ ከፍተኛ ቁጥር ያለዉ ትንኝ ጥጃና ፍየል ከሚቀመጥበት ድንኳን ተለቅሟል:: የጉልህነት መለያ መጠነ-መለከያቸዉም በጥጃና በሰዉ መካከል ($P = 0.008$) እና በፍየልና በሰዉ መካከል ($P = 0.020$) ሆኖ ተመዝግቧል:: በተመሳሳይ መልኩ በአንድ ወጥ ክፍል ዉስጥ በተቀመጡ ሰዉና ጥጃ ላይ እንደምርጫቸዉ እንድመገቡ ከተደረገ በኋላ ከተጠመዱት ትንኞች ዉስጥ በተመረመረዉ የደም ናሙና እንደተረጋገጠዉ አብዘኛዉ በጂማ አከባቢ የሚገኙ የትንኝ ዝሪያ ከሰዉ ይልቅ ከብቶችን መመገብን እንደምመርጡ ለማወቅ ተችሏል::

የምዕራፍ ፮ ዋና ዓላማ አርቅ ኬሚካሎችን ከከብቶች ከለላ (zooprophylaxis) *ጋር ማጣመር* የወባ ትንኞችን ለመቆጣጠር ያለዉን አስተዋፆ ማጥናት ነዉ፡፡ ይህ ጥናት የተካሄደዉ ቀደም ብሎ በምዕራፍ ፭ ላይ የተዘጋጀዉን የምርምር መስሪያ መስክ-መለስ ፍላት ፎርም በመጠቀም ሆኖ በተጨማሪ ሶስት አርቅ ከሚካሎችን በማካተት ነዉ፡፡ ምርምሩ በሁለት ዋና ዋና ደረጃዎች ተከፍሎ ተካህዷል፡፡ በመጀመሪያዉ ክፍል ጥናት የአርቅ ኬሚካሎች የመከላከል አቅም በላቦራቶሪ ደረጃ ተገምግሟል፡፡ የአርቅ ኬሚካሎች የመከላከል አቅም ለመለካት ሰዎች ከንዳቸዉን የእጇ ክፍል አርቅ ኬሚካል ከተቀቡ በኋላ የተራቡ የወባ ትንኝ የያዘ ኬጇ ዉስጥ ከንዳቸዉን በመክተት ትንኞች በከንዳቸዉ ላይ እስከሚያርፉ ይጠብቃሉ፡፡ የመጀመሪያዉ ትንኝ ስያርፍ እጃቸዉን ያወጣሉ ሳዓቱን ይመዘግባሉ፡፡ በሁለተኛዉ ክፍል ጥናት አርቅ ኬሚካል የተቀቡ ሰዎችን ከሌሎች አርቅ ኬሚካል ካልተቀቡ ሰዎች *ጋር ጎን ለጎን* በማስቀመጥ፤ እንዲሁም አርቅ ኬሚካል የተቀቡ ሰዎችን ከከብቶች ጎን ለጎን በማስቀመጥ የወባ ትንኞችን በመልቀቅ፤ በአንድ በኩል በአርቅዉ ኬሚካል ምክኒያት ትንኞች ከሰዉ እንድሸሹና በሌላ በኩል ደግሞ ከብቶችን በመጠቀም ትንኞች ከሰዉ ይልቅ ወደ ከብት ያላቸዉ ስቤት እንድጨምር ብሎም በድምር በሰዎችና በወባ ትንኞች *መ*ካከል ያለዉ ንክክ ለመቀነስ ታስቦ ምርምር ተደርጓል፡፡ በዚሁ መሰረት የመጀመሪያ ክፍል ጥናት ዉጤት ስናይ ለምርምር የተጠቀምንባቸዉ ሶስቱ አርቅ ኬሚካሎች ባዝ ኦፍፍ፣ ሞዚጎን እና ዲት (Buzz off, Mozigone, and DEET) አማካይ የመከላከል ጊዚያቸዉ በቅደም ተከተል 3፣61 እና 302 ደቂቃዎች ሆነዉ ተመዝግቧል፡፡ በሁለተኛዉ ክፍል ጥናት አርቅ ኬሚካል የተቀቡ ሰዎችን ከሌሎች አርቅ ኬሚካል ካልተቀቡ ሰዎች *ጋር ጎን ለጎን* በማስቀመጥ የተጠመዱ የትንኝ ቁጥሮችን በምናወዳደርበት ጊዜ ምንም ዓይነት አርቅ ቅባት ካልተቀቡ ሰዎች ድንኳን ዉስጥ የተጠመዱ ትንኝ ብዛት ሞዝጎን የተባለዉን አርቅ ቅባት ተቀብተዉ ከተኙ ሰዎች ድንኳን ዉስጥ ከተጠመደዉ የትንኝ ብዛት በ15.25 እጇ ከፍ ያለ (ጉልህነት *መ*ለያ *መ*ጠነ-*መ*ለኪያ $p < 0.001$) ከባዚ ኦፍፍ በ6.25 እጇ ከፍ ያለ (ጉልህነት *መ*ለያ *መ*ጠነ-*መ*ለኪያ $p = 0.045$) እና ከዲት በ9.75 እጇ ከፍ ያለ (ጉልህነት *መ*ለያ *መ*ጠነ-*መ*ለኪያ $p = 0.008$) ሆኖ ተመዝግቧል፡፡ በተመሰሳይ *መ*ልኩ አርቅ ኬሚካል የተቀቡ ሰዎችን ከከብት *ጋር ጎን ለጎን* በማስቀመጥ የተጠመዱ የትንኝ ቁጥሮችን በምናወዳደርበት ጊዜ ከብት ከተቀመጠበት ድንኳን ዉስጥ የተጠመዱ ትንኝ ብዛት ሞዝጎን የተባለዉን አርቅ ቅባት ተቀብተዉ ከተኙ ሰዎች ድንኳን ዉስጥ ከተጠመደዉ የትንኝ ብዛት በ11.75 እጇ ከፍ ያለ (ጉልህነት *መ*ለያ *መ*ጠነ-*መ*ለኪያ $p = 0.027$) እና ከዲት በ18.75 እጇ ከፍ ያለ (ጉልህነት *መ*ለያ *መ*ጠነ-*መ*ለኪያ $p = 0.004$) ሆኖ የተመዘገበ ስሆን ባዚ ኦፍፍ በተቀቡ ሰዎች ድንኳን ዉስጥ በተጠመዱ የትንኞች ቁጥርና ከብት በተቀመጠበት ድንኳን ዉስጥ በተጠመዱ ትንኞች ቁጥር *መ*ካከል ግን የጎላ ልዩነት አልተመዘገበም፡፡

ስጠቃለል ጥናቱ በተካሄደባቸዉ በጇማ አከባቢ ያለዉ የወባ ትንኝ ዝሪያ (mosquito population) *ፀረ-ነ*ፍሳት ኬሚካሎችን የተቋቋመ ከመሆኑም በላይ በጥናቱ ወቅት በስራ ላይ የዋሉትን ሀለቱን የወባ ትንኝ *መ*ቆጣጠሪያ ዜዴዎች ማለትም የአልጋ አጎበርና (PermaNet® 2.0) ዲዲቲ (DDT) ብቻ *መ*ጠቀም የወባ በሽታን ከአገር ለማጥፍት የተያዘዉን ግብ ማሳካት ከባድ ልሆን እንደሚችል ያሳያል፡፡ በመሆኑም የፀረ-ነፍሳት ከሚካሎች ተጋድሎ ማስታገሻ ፖሊሲ ቀርፆ በአስቸኳይ ስራ ላይ ከማዋል ጎንለጎን አማራጭ የወባ ትንኝ *መ*ቆጣጠሪያ ዜዴዎችን ማልማትና ስራ ላይ ማዋል ይጠይቃል፡ ፡ በተጨማሪም ለህዝብ የተዳረሱ የአልጋ አጎበርና ርጭት አገለግሎት በተጨባጭ የታለመላቸዉን *መ*ከላከል ደረጃ ያሟሉ ወይም አያሟሉ እንደሆነ ለማረጋገጥ ተጨማሪ ሰፋ ያለ *መ*ስክ-አቀፍ ግምገማ (large scale field trials) ብደረግ የተሻለ መረጃ ይገኛል፡፡ በዚህ የምርምር ስራ የቤት እንስሳት በተለይም የቀንድ ከብት የወባ ትንኝን በመሳብ እንደከለላ ልንጠቀመበት እንደምንችል አሳይተናል፡፡ ሆኖም ግን የተገኘዉ መረጃ በስራ ላይ ከመዋሉ በፍት በቀጣይነት ሌሎች ተያያዥ ጉዳዮች በጥናት መረጋገጥ አለባቸዉ፡፡ እነዚህም በሰዎች ማደሪያና በከብቶች በረት *መ*ካከል ልኖር የሚገባዉ አማካይ ስፍራ፤ከብቶችን በፀረ-ነፍሳት ኬሚካል *መ*ንከር በተጨማሪነት *መ*ጠናት ያለባቸዉ ጉዳዮች ናቸዉ፡፡ በአርቅ ኬሚካሎች

ግምገማ መሰረት ሁለቱ ኬሚካሎች ሞዝጎንና ባዝ ኦፍፍ ለአጭር ጊዜ ብቻ እነደምከላከሉ አሳይተናል፡፡ በአንፃራዊነት ሞዝጎን ከባዝ ኦፍፍ የተሻለ የመከላከል አቅም ያለዉ ብሆንም ሞዝጎን ገና ለማህበረሰብ አገለግሎት ላይ ያልዋለ ኬሚካል ስለሆነ ተጨማሪ ሰፋ ያለ መስክ-አቀፍ ግምገማ (large scale field trials) ብደረግ የተሻለ መረጃ ይገኛል፡፡ በመሆኑም በአሁን ሰዓት ዲት ብቻ አስተማማኝ ነፍሳት አርቅ ኬሚካል እንደሆነ እናረጋግጣለን፡፡

Curriculum Vitae

Curriculum vitae

Abebe Asale was born in 1981 in Wolaita, Southern Ethiopia. He followed his primary and secondary school in Abbela-Shoya and Soddo secondary and preparatory school respectively. He passed the Ethiopian school leaving certificate examination (ESLCE) with distinction and joined department of biology, Jimma University, Jimma, Ethiopia, in 2002. He obtained his Bachelor degree of Science in biology (BSc. In Biology) in 2005 and served as teacher in Bole Meserete-Hiwot high school, Addis Ababa, between 2005 and 2007. He joined Addis Ababa University in 2007 and graduated in masters of Science (MSc in Insect science) in 2009. Starting from 2009 he has been working in the department of biology, Jimma University at the rank of lecturer.

In 2013, he obtained a scholarship from VLIR-IUC institutional university cooperation between Jimma University, Ethiopia and Flemish Universities in Belgium.

He authored and co-authored 10 scientific papers in internationally peer-reviewed journals.

Author's bibliography

1. **Asale A**., Getachew Y., Hailesilassie W., Speybroeck N., Duchateau L. and Yewhalaw D. (2014). Evaluation of the efficacy of DDT indoor residual spraying and long-lasting insecticidal nets against insecticide resistant populations of *An. arabiensis* Patton (Diptera: Culicidae) from Ethiopia using experimental huts**.** *Parasites Vectors* 7, 131.
2. Taye B., Lelisa K., Emana D., **Asale A**. and Yewhalaw D. (2016). Seasonal dynamics, longevity, and biting activity of Anopheline Mosquitoes in Southwestern Ethiopia. *J Insect Sci.* 16, 1-7.
3. Duchateau L., Getachew Y., **Asale A**., Speybroeck N. and Yewhalaw D. (2013). Avoiding the trap of non-randomization in evaluating mosquito trapping. *Pathogens and global health*, 107, 437.
4. Yewhalaw D., **Asale A**., Getachew Y., Duchateau L. and Speybroeck N. (2013). Growing insecticide resistance and outdoor transmission: potential roadblocks for growing malaria control efforts in Ethiopia. *Pathogens and global health*, 107, 437.
5. Yewhalaw D., **Asale A**., Tushune K., Getachew Y., Duchateau L. and Speybroeck N. (2012). Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant *An. arabiensis* from South-Western Ethiopia. *Parasites Vectors* 5,159.
6. Birhanu A. and **Asale A**. (2015). Larvicidal activity of solvent extractions from some selected indigenous plants against the mediterranean fruit fly larvae *Ceratitis Capitata* identified from coffee berry (Diptera:Tephritidae) in Jimma zone, Southwestern Ethiopia. *J Appl Sci Agri.* 10, 78-85.
7. **Asale A**., Yewhalaw D., Zemene E., Alemayehu E., Tushune K., Habtewold T., Emana D. and Duchateau L. (2016). Assessing the host preference of *An. arabiensis* Patton (Diptera: Culicidae) using three alternative experimental setups in Southwestern Ethiopia. *J insect behave.* In press.
8. Berhe A., **Asale A**. and Yewhalaw D. (2016). Community perception on beekeeping practices, management and constraints in Termaber and Basona Districts, Central, Ethiopia. *Advan Agri.* doi.org/10.1155/2016/4106043
9. **Asale A.**, Tadesse M., Kebede W. and Seoyum E. (2010). Bioactivity of some essential oils against the mediterranean fruit fly (*Ceratitis Capitata*) under laboratory condition. *Pest Mgt J Eth.* 14, 40-47**.**
10. **Asale A**., Duchateau L., Zemene E., Emana D., Elemayehu E., Eba K., Habtewold T., Tefera M., Tushune K. and Yewhalaw D. (2016). Additive effect of repellent and

zooprophylaxis in malaria vector control in Southwestern Ethiopia. *Trop. Med. Int. Health*. submitted

11. Norfolk O., **Asale A**., Temesgen T., Denu D., Platts P.J., Marchant R. and Yewhalaw Y. (2016). Diversity and composition of tropical butterflies along an Afromontane agricultural gradient in the Jimma Highlands, Ethiopia. *Biotropica* In press.